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A LABORATORY APPROACH TO THE INVESTIGATION AND EVALUATION OF HEMOLYTIC MECHANISMS*

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After investigating the literature and inquiring of other hematology laboratories, we have concluded that the laboratory approach to a hemolytic problem is neither adequate nor consistant. Because of the multiplicity of tests in the literature, the technologist is often required to spend hours running random procedures in the hope that one abnormality will be demonstrated. However, tests done on successive days cannot be correlated one with the other. The problem is further complicated by the fact that the usual standard "screening" procedure of the reticulocyte count and bilirubin determination cannot be used to fully rule out the presence of a hemolytic process. Being aware of this problem we set out to find a series of tests that would be sufficient as well as practical.

At this point it is well to distinguish between a hemolytic anemia and a hemolytic disease process. It is preferable to speak of hemolytic disease either with or without anemia rather than hemolytic anemia alone since it is recognized that anemia is a result of a pathologic mechanism causing an abnormally rapid destruction of erythrocytes. If the bone marrow can keep up with this destruction, no anemia results. If not, the patient becomes anemic.[1] Thus as Crosby suggests, we may speak of either "compensated" or "decompensated" hemolytic anemia.[2]

When hemoglobin is degraded normally this destruction takes place intracellularly within the phagocytic cells of the reticulo-endothelial system,[3] e.g., within the spleen. The iron radical is returned to the "iron pool" to be reutilized and the resulting porphyrin fraction is changed into bilirubin which is bound to protein, principally albumin. Then it is carried by the blood stream to the liver where it combines with glucuronic acid. This conjugated bilirubin is then excreted into the bowel by way of the bile. Due to the action of intestinal bacteria, the bilirubin is further acted on in the bowel resulting in various breakdown products collectively referred to as "fecal urobilinogen." The terms "direct" and "indirect" bilirubin refer to the behavior of the pigments in the Van den Bergh reaction. Bilirubin gives an "indirect" reaction until it has undergone metabolism in the liver. Therefore, excessive hemolysis is often

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accompanied by hyperbilirubinemia of the "indirect" type.[1,4] Urobilinogen, since it is also reabsorbed from the bowel, may be detected in the urine in the presence of hemolysis. Even though the study of fecal and urinary urobilinogen as an index of hemolysis has been suggested, other variable factors such as the total amount of circulating hemoglobin and the excretory function of the liver and kidneys, make these tests difficult to evaluate.[5] It should be remembered that hemolysis may occur without significant elevations in either serum bilirubin, urine or fecal urobilinogen.[1]

Abnormal hemolysis may also occur in the circulating blood as well as in the tissues. Hemoglobin released into the plasma causes a different series of events to take place, apparently depending upon the amount of hemoglobin liberated. If hemoglobin levels in the plasma are less than 100-135 mg.%, the haptoglobins appear to bind the hemoglobin molecules forming haptoglobin-hemoglobin complexes. These haptoglobins are believed to be alpha or beta globulins and can be detected by electrophoresis.[6,7] Some of the liberated hemoglobin will also combine with the serum albumin to give a compound known as methemalbumin. This pigment can be detected by the hemochromogen it forms when plasma is mixed with ammonium sulfide. (Schumn's Test)[8] If the concentration of liberated hemoglobin is great enough, the plasma fractions will become saturated, and the free hemoglobin is then excreted in the urine.[6]

Because the red cell life span is shortened in hemolytic diseases the best single method of detecting hemolysis is the determination of the red cell survival time. The use of radio-chromium (Cr^{51}) as a red cell tag is relatively easy and reliable. The shortening of the half-life to approximately 20 days from the normal of 29-31 days indicates abnormal hemolysis.[9] The decreased half-life may not necessarily be accompanied by increased pigment metabolism since, as Crosby[1] states, "the plasma bilirubin may be normal in hemolytic disease." Radio-chromium tagging has not been widely employed as a routine procedure since the method does require special equipment as well as radio product authorization.

The exact hemolytic mechanism in a given patient apparently depends upon the nature of the cell defect and the anatomical site of hemolysis as pointed out above. Defective erythrocytes may be formed in the bone marrow and released into circulation. Their defect, either an abnormal hemoglobin or defective stroma, renders them more susceptible to destruction by the spleen or by the mechanical trauma of buffeting about in the circulation. This type of hemolytic disease is termed "intrinsic" since the defect lies within the cell itself. The congenital hemolytic diseases as a class are included in this group.

On the other hand, normal erythrocytes delivered into peripheral circulation may become coated with an abnormal antibody in the plasma and as a result, become more susceptible to overdestruction. This mechanism is referred to as "extrinsic" hemolysis since the defect lies outside the cell. The acquired hemolytic diseases are included in this group.[5]

Methods:

The purpose of this paper is not to present the techniques involved in hemolytic determinations since they may be found in the references, but to define their usefulness. In a few instances minor modifications in

specific methods have been made and noted. In our attempt to determine the nature of hemolytic mechanisms we have utilized the following series of procedures:

1. Wright's stained blood smear
   A study of the morphological appearance of red cells often gives a clue as to the possibility of a hemolytic anemia. The presence of irregular red cell shapes such as spherocytes, elliptocytes, or meniscocytes is usually evident.
2. Detection of abnormal hemoglobins
   In order to detect abnormalities of hemoglobin, electrophoresis[10] is carried out and, if needed, alkali denaturation[11] and solubility studies can be utilized. The use of a reducing agent such as sodium metabisulfite is helpful in detecting the presence of sickle cells but is valueless in differentiating sickle cell disease vs. sickle cell trait.[12]
3. Reticulocyte count[13]
   A reticulocyte count is usually indicative of bone marrow compensatory activity but, as Wasserman[18] et al. have shown, reticulocytosis is not always present in hemopathic hemolytic diseases such as leukemia, uremia, and infection. We prefer New Methylene Blue, color index 927 (Brecker's Method),[13] to brilliant cresyl blue as a reticulocyte stain. It is relatively simple to use, renders the reticulocytes easily discernible, and yields reproducible results.
4. Plasma hemoglobin[14]
   An increased plasma hemoglobin denotes increased intravascular hemolysis. Whereas a normal plasma hemoglobin will rule out increased intravascular hemolysis, it will not rule out extravascular hemolysis. It is believed that a measure of plasma hemoglobin includes both free hemoglobin and the various hemochromogens which are produced in intracellular hemolysis.
5. Autohemolysis test[14]
   The autohemolysis test detects spontaneous lysis when cells are allowed to incubate in their own serum under sterile conditions at 37° C. The amount of lysis after 24 hour incubation and again after 48 hour incubation is compared to an initial sample. The test is rather non-specific since certain intrinsic and extrinsic hemolytic diseases may show accelerated autohemolysis. As Dacie[14] states, "if hemolysis is accelerated, the observer is entitled to consider it as a valuable pointer to a hemolytic process, but nothing more."
6. Fragility studies
   Cell defects can at times be differentiated by subjecting cells from a given patient to a series of stresses which will reveal abnormalities in their tensile strength.[15] The use of the osmotic and mechanical fragilities are helpful in this regard.
   a) Osmotic fragility[14]
      When erythrocytes are placed in descending dilutions of hypotonic sodium chloride, a certain number of them will hemolyze. A measure of this hemolysis is an index to the resistive power of the cells to the hypotonic saline solutions. To be more exacting in the detection of variations from normal, we used saline solutions descending in hypotonicity of 0.05% rather than 0.1% in the range from initial to complete hemolysis. With the hope

of augmenting the difference between normal cells and cells with intrinsic defects, we routinely incubated blood for this test for 24 hours at 37° C under sterile conditions. As Dacie[14] points out, in certain mild congenital hemolytic anemias, abnormal osmotic fragility, not detected before incubation, becomes apparent after incubation.

b) Mechanical fragility[16]
Erythrocytes are susceptible to mechanical trauma and may be readily lysed in vitro by rotation at a slow speed in an Erlenmeyer flask containing several small glass beads.[16] We modified this test by allowing sterile incubation at 37° C for 24 hours. However, Young[17] reports that differences between normal and spherocytic cells after such incubation are less significant in the mechanical fragility than in the osmotic fragility test. At the present time data are not available as to the use of the mechanical fragility in many disease states. Spherocytes, sickled cells, and agglutinated corpuscles may show increased mechanical lysis.[14]

7. Antibody Studies
The detection of extrinsic hemolytic mechanisms depends upon the demonstration of abnormal plasma or serum constituents. At the present time this detection consists of a search for abnormal antibodies. Even though this group is not clearly understood, we can isolate certain types of antibodies which often produce hemolytic diseases.

a) Coombs' tests[14]
The Direct Coombs' test is used to demonstrate antibodies which have become fixed to patient's cells in vivo. The Indirect Coombs' test demonstrates abnormal antibodies in the patient's serum which can be absorbed onto normal test cells and cannot be removed by washing. Both tests are frequently positive in acquired hemolytic diseases. The Direct Coombs' may remain positive during remission whereas the Indirect Coombs' may become negative. As Dacie points out, the presence of free antibodies in the serum is probably correlated with the active hemolytic process. The Direct Coombs' may be positive in erythroblastosis fetalis and in rare blood antigens.[14]

b) Detection of incomplete antibodies using trypsinized erythrocytes[14]
Since it has been shown that enzyme-treated test cells may be more sensitive to various antibodies we routinely "sensitized" the test cells with trypsin in order to enhance the in vitro demonstration. This test was done in addition to the Coombs' since positive results with negative Coombs' have been reported.[14]

c) Detection of "warm" and "cold" antibodies[14]
Other antibodies, especially against the Rh group, may be detected in the patient's serum. These antibodies, on the basis of their behavior in vitro, may be divided into "warm" and "cold" groups. A screening procedure for these was carried out at 4° C and 37° C[14] using patient's serum and test cells in both saline

and albumin since some are "'complete," functioning against red cells in a saline solution, and others are "incomplete,"[5,14] requiring a protein or high molecular weight medium.[18]

Results:

A control was tested along with each patient under study. Healthy individuals with normal hemoglobins selected from laboratory and student personnel or pre-operative patients exhibiting no apparent hemolytic disease, were chosen. From the results obtained on forty eight out of fifty of these individuals our range of normal values were established as listed below.

Plasma hemoglobin 1.0-6.0 mg. %
Reticulocyte count 0.5-2.0 %
Autohemolysis test 0-0.6% lysis after 24 hours at 37° C
0.4-3.5% lysis after 48 hours at 37° C
Osmotic fragility after 24 hours at 37° C
initial lysis 0.65-0.5% NaCl
complete lysis 0.4-0.2% NaCl
Mechanical fragility after 24 hours at 37° C
1.0-7.0% lysis

Patients exhibiting intrinsic cell defects were divided into two groups according to the presence or absence of abnormal hemoglobins. Results of these studies are shown in Tables I and II.

TABLE I—Findings in Intrinsic Hemolytic Diseases with Abnormal Hemoglobins

HGB. Type	Plasma HGB mg. %	Retic. Count %	AUTOHEMOLYSIS % lysis		OSMOTIC FRAGILITY 24 hr. at 37°C % NaCl		MECHANICAL FRAGILITY 24 hr. at 37°C % lysis
			24 hr.	48 hr.	Initial	Complete	
S-S	3.8	5.2	0.4	...	...	...	...
S-S	6.9	2.6	0.6	0.7	0.55	0.2	9.4
S-S	43.0	18.0	0	0.8	0.85	H20	16.0
S-S	60.0	13.1	0.6	...	1.0	H20	13.0
S-C	4.2	18.0	2.1	3.5	1.0	H20	2.0
C-C	3.2	7.6	0.5	2.9	0.65	0.2	7.9
Normal	1.0-6.0	0.5-2.0	0-0.6	0.4-3.5	0.65-0.5	0.4-0.2	1.0-7.0

In patients with homozygous S hemoglobin, autohemolysis was not accelerated even though the mechanical fragility was increased. Two patients demonstrated the characteristic resistant osmotic fragility and one showed a questionable resistance. A typical case is shown in Figure 1. The one case of hemoglobin S-C showed accelerated autohemolysis at 24 hours, resistant osmotic fragility, and normal mechanical lysis. The significant finding in the case of homozygous C hemoglobin was the slightly elevated mechanical fragility despite normal autohemolysis and osmotic fragility. Increased plasma hemoglobin values in these cases seem to indicate that a certain amount of hemolysis in such hemoglobinopathies occurs intravascularly. The reticulocytosis found probably indicates bone marrow response to the hemolysis.

From Table II it is evident that all cases which showed spherocytosis on the peripheral smear had corresponding accelerated autohemolysis and increased osmotic fragility. However, by our method, mechanical fragility values fell within normal range. The increased plasma hemoglobins indicate that spherocytes are probably susceptible to intravas-

TYPICAL FINDINGS IN SICKLE CELL DISEASE
AS COMPARED WITH NORMAL FINDINGS

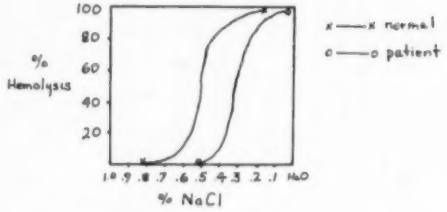

Figure 1

TYPICAL FINDINGS IN CONGENITAL SPHEROCYTOSIS
AS COMPARED WITH NORMAL FINDINGS

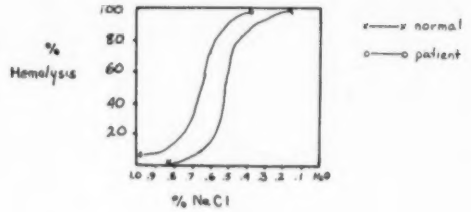

Figure 2

TABLE II—Findings in Intrinsic Hemolytic Diseases Without Abnormal Hemoglobins

Case No.	Plasma HGB mg. %	Retic. Count %	AUTOHEMOLYSIS % lysis		OSMOTIC FRAGILITY 24 hr. at 37°C % NaCl		Mechanical Fragility 24 hr. at 37°C % lysis	Red Cell Shape
			24 hr.	48 hr.	Initial	Complete		
1.....	13.6	...	1.4	23.0	0.65	0.2	3.7	spherocytes
2.....	7.3	...	1.1	8.4	0.75	0.3	0	spherocytes
3.....	12.6	...	1.0	24.0	0.75	0.3	6.0	spherocytes
4.....	66.0	18.0	2.7	8.5	1.0	0.1	5.9	spherocytes
5.....	10.0	12.0	4.2	...	0.85	0.35	5.5	spherocytes
6.....	3.5	10.1	1.1	...	1.0	0.35	2.5	spherocytes
7.....	...	2.8	0	...	0.55	0.3	...	normal
8.....	10.0	1.7	0.3	4.3	1.0	0.1	7.0	normal
9.....	5.3	1.0	0.7	...	0.85	0.2	13.0	normal
10.....	...	10.0	1.5	...	0.75	H20	2.2	normal
11.....	3.2	12.0	2.0	18.0	0.85	0.2	0	normal
12.....	1.0	2.3	0.3	0.6	1.0	0.1	12.0	normal
13.....	2.6	10.7	1.0	4.0	0.75	0.1	0	normal
14.....	...	1.5	7.0	...	1.0	0.4	17.0	normal
15.....	...	11.4	0.6	5.8	1.0	H20	8.0	normal
16.....	1.0	4.8	0	...	0.55	0.4	14.0	ovalocytes
Normal.....	1.0–6.0	0.5–2.0	0–0.6	0.4–3.5	0.65–0.5	0.4–0.2	1.0–7.0	Normal

cular lysis. A typical case is shown in Figure 2.

The patients exhibiting a cell defect not evident morphologically are more difficult to interpret. Plasma hemoglobins were normal except in case no. 8. Autohemolysis at both 24 and 48 hours as well as mechanical fragility showed no definite pattern. Osmotic fragility was increased in all cases with cases 8, 10, 12 and 13 showing resistant "tailing." Case 8 is a patient with non-spherocytic hemolytic jaundice who had undergone splenectomy approximately ten years previously with no remission. Since splenectomy she has maintained her hemoglobin at a level of 4-6 gm. %. Case no. 7 is the asymptomatic father of case no. 8. The only significant finding in the case of ovalocytosis was the increased mechanical fragility

As indicated in Table III five cases of extrinsic hemolytic disease are presented. In no case could we detect accelerated autohemolysis. In cases no. 2 and no. 3 the osmotic fragility was slightly resistant. The high plasma hemoglobin demonstrated in case no. 5 is apparently the result of the anti-B antibody acting on the patient's AB cells as a result of the incompatible transfusion. The antibodies detected in this case seem to have had no significant effect on the autohemolysis or fragilities.

TABLE III—Findings in Extrinsic Hemolytic Diseases

Type of Extrinsic Hemolytic Disease	Plasma HGB mg. %	Retic. Count %	AUTOHEMOLYSIS % lysis		OSMOTIC FRAGILITY 24 hr. at 37°C % NaCl		ANTIBODY DEMONSTRATED					
									4°C		37°C	
			24 hr.	48 hr.	Initial	Complete	Coombs	TRYP.	Sal	Alb	Sal	Alb
Idiopathic acq	...	...	0	..	0.65	0.2	1:4096	—	—	—	—	—
Idiopathic cold	...	...	0.6	0.6	0.35	0.2	—	—	+	—	—	—
Metastatic	...	...	0.4	0.7	0.45	0.2	—	—	+	+	+	+
Drug induced	3.5	15.6	0.5	...	...	..	—	—	+	+	—	—
Incompatible transfusion	45.5	50.4	0.1	1.2	0.75	0.2	1:128	+	—	—	—	—
Normal	1.0–6.0	0.5–2.0	0–0.6	0.4–3.5	0.65–0.5	0.4–0.2	—	—	—	—	—	—

Note: Mechanical fragility on case 5 was normal.

Table IV includes three groups of patients with severe anemia who were initially suspected of having hemolytic disease. Pernicious anemia is included here although it is well recognized as a hemolytic disease involving a deficiency in the red cell itself as well as exhibiting an abnormal plasma factor. Case no. 1 was combination iron deficiency as well as pernicious anemia since blood was detected in the stool on several occasions. The only consistant finding in these three cases is the increased osmotic fragility. Autohemolysis is accelerated in two of the three patients.

Three patients with iron deficiency anemia are presented. Each case exhibited a resistant osmotic fragility which is consistant with the cases studied by Ham[16] in which he demonstrated the same resistant pattern following sterile incubation for 24 hours at 37° C. Two showed slightly increased mechanical fragility.

The findings in the two cases of aplastic anemia are not felt to be significant. However, the case of benzene poisoning at the time of study was apparently hemolyzing intravascularly without the expected reticulocytosis as evidenced by the high plasma hemoglobin and the normal reticulocyte count.

In addition to the cases presented in the tables, three cases of leukemia in our study demonstrated hemolytic episodes. One chronic myelocytic showed an increased osmotic fragility with no excess autohemolysis. Two chronic lymphatics developed a positive Indirect Coombs along with a positive trypsin test, one demonstrating low titer antibodies at 4° C in saline and albumin.

TABLE IV—Findings in Various Dysplastic Anemias

Type of Anemia	Plasma HGB mg. %	Retic. Count %	AUTOHEMOLYSIS % lysis		OSMOTIC FRAGILITY 24 hr. at 37°C % NaCl		MECHANICAL FRAGILITY 24 hr. at 37°C % lysis
			24 hr.	48 hr.	Initial	Complete	
Pernicious Anemia							
1	33.5	1.3	0	..	1.0	0.3	1
2	4.6	5.4	6.0	..	1.0	0.1	16.0
3	...	1.3	3.0	3.5	1.0	0.2	6.0
Iron Def. Anemia							
1	5.7	46.0	0	1.2	0.35	0.1	9.0
2	7.7	1.8	0.3	0.7	0.65	0.1	2.2
3	...	1.4	0.5	4.1	0.75	H20	8.0
Aplastic Anemia							
Previous transfusion	...	...	0.5	0.8	0.55	0.35	...
Benzene Poisioning	8.0	0.9	0.5	1.3	0.55	0.1	12.3
Normal	1.0–6.0	0.5–2.0	0–0.6	0.4–3.5	0.65–0.5	0.4–0.2	1.0–7.0

Discussion

As Wasserman[18] et al. have reported, the use of red cell survival studies has shown a decreased erythrocyte life span in many conditions in which hemolysis, as usually defined, is not a prominent feature. This group includes certain infections, chronic hepatic and renal diseases, malignancies and many blood dyscrasias. Since these differ in most respects, both clinically and hematologically, from diseases showing characteristics of well recognized hemolytic syndromes, e.g.. sicklemia, spherocytosis, acquired hemolytic diseases, etc., they have suggested the

inclusive term "hemopathic hemolytic anemia" for these hemolytic syndromes, as distinct from such symptomatic hemolytic anemias.

The usual criteria of reticulocytosis and hyperbilirubinemia in the detection of a hemolytic process will fail to detect this hemopathic hemolytic group. Therefore it is obvious that a different screening series of tests be used to detect every case of hemolysis. The use of radiochromium tagged red cells appears to be the best method for the detection of hemolysis in any case. The tests presented in this paper appear to be useful in an evaluation of the nature of the hemolytic mechanism in either of the two groups.

One advantage of a series of such tests is the fact that adequate blood-samples can be drawn with one atraumatic venepuncture. Then, if necessary, blood transfusions or other forms of therapy can be initiated. These tests then can be correlated with each other.

The nature of the hemolytic mechanism in sickle cell disease appears to be similar in every case studied. Cells from these patients exhibited characteristic resistant osmotic fragilities and increased mechanical lysis in vitro. It is possible by means of these studies that various patterns may be characteristic of the other hemoglobinopathies. Our results are suggestive in this regard but further studies are necessary. Mechanical fragility appears to be increased in sickle cell disease but is questionable in hemoglobin C disease.

Hemolytic mechanisms in the non-spherocytic hemolytic diseases are more difficult to evaluate since the positive findings cannot be correlated with each other. Modifications of the autohemolysis test by the addition to the blood prior to incubation of certain metabolizable substances such as glucose or other substances known to be good blood preservatives, may help in differentiating these defects.[19]

Detection of acquired hemolytic mechanisms depends upon the use of serological tests for abnormal antibodies. Since the action of these antibodies is directed against the patient's own red cells, the detection of accelerated autohemolysis and increased lysis as a result of stresses in vitro, is helpful in an evaluation of the nature of the hemolytic process. The mechanism by which the antibody brings about actual hemolysis may become more apparent by means of these studies.

Summary

1. Measurement of plasma hemoglobin was found helpful but not diagnostic in the investigation of certain hemolytic states. Normal and increased values were found in each group studied. Further investigation of the plasma for methemalbumin and hemoglobin binding plasma fractions is indicated.
2. Normal reticulocyte counts cannot be used to rule out the presence of hemolytic process. A normal rate of autohemolysis was exhibited by five of the six hemoglobinopathies, the five cases of extrinsic hemolytic disease, four of the nine cases of non-spherocytic hemolytic disease, and the case of aplastic anemia. Increased values were obtained in all six cases of spherocytosis. Accelerated autohemolysis is usually evident at 24 hours. Out of eleven cases exhibiting normal 24 hour autohemolysis, three showed acceleration at 48 hours.
3. Osmotic fragility studies were helpful in detecting non-spherocytic hemolytic syndromes as indicated by the increased values obtained

in each of the eight cases presented. Its value is questionable in the acquired hemolytic diseases since two of the four cases in which this test was done exhibited slight resistance. Consistantly increased resistance was noted in all cases involving hemoglobin S, and consistently increased hemolysis was noted in each case of spherocytosis. The increased resistance shown by the iron deficiency cases makes the detection of this anemia, in association with other hemolytic processes, important.

4. Mechanical fragility studies seem to be helpful in the evaluation of cell defects. Four of the five cases of abnormal hemoglobins showed increased lysis. Normal mechanical lysis was obtained in the cases of spherocytosis. The variable results obtained in the non-spherocytic cases could not be correlated with the other positive findings. Further studies of mechanical lysis in extrinsic hemolytic processes needs to be carried out.

Conclusions

1. A battery of tests for the evaluation of hemolytic mechanisms has been presented along with results obtained in diseases of both intrinsic and extrinsic cell defects.
2. At the present time the best single method for the detection of hemolysis appears to be the use of red cell survival studies.
3. The differentiation of the intrinsic vs. extrinsic hemolytic mechanisms depends upon the utilization of pigment metabolism studies, abnormal hemoglobin studies, fragility patterns, and the detection of plasma or serum antibodies.

BIBLIOGRAPHY

1. Crosby, W. H., The Metabolism of Hemoglobin and Bile Pigments in Hemolytic Disease, American Journal of Medicine, 18: 112, January, 1955.
2. Crosby, W. H., The Hemolytic States, Bulletin of the New York Academy of Medicine, 30: 27, January, 1954.
3. London, I. M., Metabolism of Hemoglobin and of Bile Pigment, Bulletin of the New York Academy of Medicine, 30: 509, July, 1954.
4. Billings, B. H. and Lathe, G. H., Bilirubin Metabolism in Jaundice, American Journal of Medicine, 24: 111, January, 1958.
5. Dameshek, W., Hemolytic Anemia, Direct and Indirect Indications, Pathogenetic Mechanisms and Classifications, American Journal of Medicine, 18: 315, February, 1955.
6. Allison, A. C. and Rees, W., The Binding of Hemoglobin by Plasma Proteins (Haptoglobins), British Medical Journal, p. 1137, November 16, 1957.
7. Lathem, W. and Worley, W. E., The Transport of Hemoglobin in Plasma, Clinical Research, 6: 12, January, 1958.
8. Fairley, N. H., Methemalbumin, Part I, Clinical Aspects, Part II, Its Synthesis, Chemical Behavior and Experimental Production in Man and Monkeys, The Quarterly Journal of Medicine (New Series), 10: 95, April, 1941.
9. Weinstein, I. M. and LeRoy, G. V., Radioactive Sodium Chromate for the Study of Survival of Red Blood Cells, II, The Rate of Hemolysis in Certain Hematologic Disorders, The Journal of Laboratory and Clinical Medicine, 42: 368, September, 1953.
10. Durrum, E. L., A Microelectrophoretic and Microionophoretic Technique, The Journal of the American Chemical Society, 72: 2493, July 20, 1950.
11. Singer, K., Chernoff, A. I. and Singer, L., Studies on Abnormal Hemoglobins, Blood, 6: 413, May, 1951.
12. Diggs, L. W., The Morphology of Human Blood Cells, first edition, Philadelphia, W. B. Saunders Co., 1956.
13. Brecker, G., New Methylene Blue as a Reticulocyte Stain, American Journal of Clinical Pathology, 19: 895, September, 1949.

14. Dacie, J. V., The Hemolytic Anemias, Congenital and Acquired, first edition, New York, Grune & Stratton, 1954.
15. Ponder, E., Hemolysis and Related Phenomena, first edition, New York, Grune & Stratton, 1948.
16. Ham, T. H., A Syllabus of Laboratory Examinations in Clinical Diagnosis, fifth edition, Cambridge, Harvard University Press, 1955.
17. Young, L. E., Hereditary Spherocytosis, American Journal of Medicine, 18: 486, March, 1955.
18. Wasserman, L. R., Stats, D., Schwartz, L., and Fudenberg, H., Symptomatic and Hemopathic Hemolytic Anemia, American Journal of Medicine, 18: 961, June, 1955.
19. Young, L. E., Izzo, M. J., Altman, R. I., and Swisher, S. N., Studies on Spontaneous in vitro Autohemolysis in Hemolytic Disorders, Blood, 11: 977, November, 1956.

SCIENCE INFORMATION*

Syphilis, once believed to be a disappearing disease, has been increasing since 1955, according to the publication "Patterns of Disease," prepared by Parke, Davis & Company for the medical profession. It points out that a rise of about 15 percent occurred in the reported case rate of late and late latent syphilis from 1955 to 1957. In general, however, the incidence of syphilis has decreased by 82 percent since a peak incidence in 1943, when penicillin therapy was introduced. The incidence of gonorrhea has shown a less dramatic drop—54.5 percent—since a peak year in 1947, but, unlike syphilis, gonorrhea has continued to decline.

* * * * *

Successful control of syphilis depends not merely on treating its victims but on tracking down and treating the hidden sources of infection. One study is cited where investigation of the contacts of a single patient with early latent syphilis uncovered a chain of infection involving 326 persons. Of all of those involved, 72 were found to have syphilis and 65 were potentially infective. This occurred, too, in an area supposedly free from infectious syphilis.

Over a three-month period last year, interviews with 784 patients with primary and secondary syphilis led investigators to more than four times as many contacts—3,195 persons who might have been responsible for the infection or contracted it from the patients.

* * * * *

Venereal disease, still a major health problem, is far commoner among men than among women. Evidence for this statement is presented in the current issue. Last year, the reported incidence of syphilis was 30 percent higher among men than women and that of gonorrhea more than 140 percent higher. Part of the reason for this difference, however, may be the fact that, in man, signs of veneral disease are more obvious and symptoms more painful, "Patterns" points out.

* * * * *

The incidence of veneral disease among Americans under 20 is increasing. Every day 136 cases are reported for persons in this age group—that is one case every 11 minutes.

During the past two years, "Patterns" points out, more than half of reported cases of gonorrhea and syphilis were among teenagers and young adults, although this age group comprises only 13 percent of the total population.

Peak reported incidence of venereal disease among men occurs at the age of 23, among women at 18.

* * * * *

There are close to two million persons with syphilis in this country, and each year there are one million new cases of gonorrhea. The reported incidence of gonorrhea is over 30 times greater for nonwhite persons than for the white population, although it has been suggested that this tremendous difference may be due in part to under-reporting in the white population.

* * * * *

Most cases of reported syphilis are diagnosed in the late stages. Out of a total of 135,542 cases reported last year, more than 100,000 were late and the late latent syphilis as opposed to 20,346 cases of early latent syphilis.

From 1955 to 1957, a rise of about 15 percent has occurred in the reported case rate of late and late latent syphilis, indicating that many cases of early syphilis are either not recognized or inadequately treated.

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* From Science Information Bureau, 445 Park Ave., New York 22, N. Y., the publication, "Patterns of Disease," prepared by Parke-Davis & Company for the medical profession.

THE CONTROL OF ACCURACY IN THE LABORATORY†

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Some time ago I attended a Workshop on Instrumentation sponsored by the New England Society of Pathologists. Many problems of accuracy control were covered. I realize that *your* laboratory never renders inaccurate reports. For the sake of your fellow technologists in that "other" laboratory, however, let me present some of the thoughts that I brought back from that meeting.

In the first place, what is "accuracy"? It is many things to many people. The "norther,"* when predicted for Houston, often does not go beyond Dallas. This error may lie well within the loose limits of accuracy imposed upon the weather bureau by fickle skies. At the other extreme we know of the careful doctor who wishes to be quite certain that Mr. Smith's cholesterol is 260 mg.% as reported, and not 255 mg.% or 265 mg.%. When confronted with the facts of inevitable limitations of accuracy, the doctor listens to the pathologist with a kindly, slightly pained smile. As a true practitioner of this most humanitarian of all sciences, the doctor is quite tolerant toward the laboratorian's discourse. To keep up the pathologist's morale, valuable time is sacrificed. In the end, however, the doctor makes his point quite clear: For the sake of his patient, absolute accuracy is mandatory. There can be no compromise. He informs the pathologist of the intent to obtain an unequivocal result, in this laboratory or somewhere else. Needless to say, he will find the happy laboratory where results are reported on the dot, unhampered by inquiry into the inherent error of the method.

Somewhere between these extremes lie the requirements of our daily operations. Let me try to list their pitfalls.

Personal Attitude

You all know the salesman, who, after having sold you a "best buy" in life insurance, cordially pats your back and begins to call you by your first name. Having thus demonstrated his intimate concern for your welfare, he is ready to sell you a second policy. Should you indicate the intent of studying said policies critically, your benefactor will argue in the spirit of deep personal offense; you have questioned his knowledge as well as his intent to offer you the best man has to give to his fellow man.

Believe it or not, there are some such salesmen among pathologists. And, if I may share a confidence with you, *even technologists* have been known to follow that approach.

These people are very nice to deal with. They learn quickly as they never attempt to learn very much. Their manner is self-assured and their conversation informed. As they have never weighted down their memory with the thorough knowledge of any one procedure, they carry at their finger tips fragmentary information on all that is to be known.

Judging by my experience, little is to be feared from the stupid or the openly ignorant. They are easily discovered. It is difficult, however, to guard against those talented amateurs whose smoothness is but a cover-up for incompetence.

† Received for publication May 1958. From an address presented before the Texas Society of Medical Technologists, April 26, 1958.

* Local term for northwind.

Procedure

Everyone will agree that all who perform a procedure should be thoroughly familiar with it. At the same time, there exist considerable differences in peoples' concept of "familiarity." To my mind, proper performance of a test requires both the knowledge of the technical aspects of a procedure and an understanding of its theoretical foundations. Let me illustrate this point with a perfect example of "unfamiliarity" with a procedure. A young lady of my acquaintance had just assumed a position as bacteriologist in a hospital laboratory. As one of her duties, she was to do bacterial counts on milk samples for public health purposes. As you know, this procedure has been standardized by the American Public Health Association. It consists in pour plate counts from several dilutions of each sample. At least four sterile pipets are needed for each series of dilutions. When preparing for her first milk analysis, the bacteriologist found all of one pipet for that purpose. On inquiry, a senior member of the laboratory staff encouraged her to proceed with one pipet. He explained to her that this was not a research laboratory. The bacteriologist's stubborn refusal to cooperate brought the director of the laboratory to the scene. This pathologist, in recognition of his serious responsibility, quickly solved the problem: He suggested to rinse the pipet with formalin between transfers. This proposal was turned down by the bacteriologist. She ultimately obtained pipets. Her counts immediately detected samples which exceeded the permissible range of bacterial contamination. No such observations had been made during the tenure of the preceding bacteriologist. I might add that the competent technologist did not win a popularity contest.

Performance

The open discussion of a worker's performance is frowned upon by individuals of tact and social grace. This is a very good rule of communal living. Tactfulness should not, however, exclude the frank evaluation of a worker by her or his supervisor. When a problem in this area is tackled personally between two parties, progress can usually be made. When, on the other hand, a supervisor voices complaints to everyone other than the technologist in question, fireworks are in the making.

Pipetting, for example, becomes a personal habit. It can become a good habit or a bad habit. Grossly faulty technique is easily detected. Lack of precision, or loss of precision, on the other hand, may become apparent only by objective testing. Test methods are simple. It is difficult, however, to convince technologists of several years experience of the need for checks on their technique. The problem is purely psychological. The obstacles are misplaced pride, annoyance at "unnecessary" botheration, and fear of failure.

Glassware

Various points of view determine the selection of glassware. Purchasing agents like to save money. Young ladies with aesthetic inclinations are swayed by delicate shapes and pretty black and red markings. Worried pathologists look for the manufacturer's statement of minimal tolerances. Unfortunately, it is not at this time possible to obtain consistently accurate glassware, regardless of the method of choice. The

laboratories which have kept records of their calibrations present some very illuminating data. At the New Orleans Charity Hospital[1] 20% of blood sugar tubes were found to be unsatisfactory. One of these tubes was 20% too large! Of the best tubes that money could buy, 10% were still unsatisfactory. The best Sahli pipets may vary ±5%.[2] In Ellerbrook's laboratory[2] a group of WBC pipets was found which gathered very well around a mean. This mean, however, was 11% too high, indicating that the pipets were 11% too small. Annino[3] encountered pipets with errors as high as 17%. He found no consistency in the number of inaccurate pipets from one batch to the next.

In the light of these observations, it is difficult to maintain the attitude that "most" glassware is "good enough" for clinical work.

Standards

A standard is thought by some to be a solution which, once it has been placed in the properly labeled bottle, becomes endowed with a magic quality of infallibility. Unfortunately, standards do not know of the high esteem in which they are held. They may change, particularly after they have been diluted.

Instruments

Our laboratory equipment rapidly approaches the best Rube Goldberg ever imagined. To those with a love for turning knobs and observing dials, chemistry has become a happy experience. Yet, advances in instrumentation call for their dues. When automatic transmissions were placed in automobiles, someone commented that cars had become very easy to drive but very hard to repair. This statement applies, *mutatis mutandis*, to laboratory instruments. The operation of even complicated electronic equipment calls for no more than a knowledge of English sufficient to read relatively simple directions, and for enough fingers to turn knobs, position switches, and push buttons. Maintenance, on the other hand, may some day call for Ph.D.'s in electronics in the place of biochemists. As an example let me enumerate the elements which can go out of order in a relatively simple photometer such as the Klett.

Bulb (loss in light intensity, improper positioning).
2 Photo-electric cells (natural aging, corrosion by spilled solution).
Galvanometer.
Galvanometer switch.
Potentiometer
Filters (filters of similar # have been found to vary as much as 5% in transmittance. A curve prepared with one filter may, therefore, be incorrect when used with another filter of the same #).

Calculations

A knowledge of arithmetic is quite essential in this age of mathematics. It helps to know a little algebra; and the worker who can manipulate logarithms will forever acquire the reputation of being an "egg head." In addition, common sense remains an invaluable aid in our endeavors. When, for example, urine reads with the specific gravity of water (namely 1.000) it is better to check the urometer than to render a report. Similarly, it is unwise to report CO_2 combining power of over 100 vol. %. The doctor may remember that, by definition, percent values can not exceed 100.

Discussion

Having paraded the pitfalls of our daily operations before my mental eyes, I stand in awe. I hope that you will join me. Gone are the days when, as a medical student, I performed RBC, WBC, differential counts, and Sahli hemoglobin determinations. Having gone through these procedures with what appears today almost childish seriousness, I believed I knew something about the patient's blood. Nowadays, we rarely do RBC counts because of the high sampling error of the method; for the same reason we consider WBC and differential counts as no more than crude indicators of numerical changes in cell populations. We would be embarrassed at the mere thought of relying on the Sahli hemoglobins, and have found it necessary to add the hematocrit to our routine blood determinations because of accuracy and reproducibility of this method.

Surely, changes of this type represent progress. Yet, I fear that the means of progress have climbed ahead of us, the human beings who are supposed to control them. Machines will usually give us answers. They will not tell us, however, whether these are the correct answers. That final decision we must make ourselves.

Anatomists tell us that every human being has a brain. To be sure, there is some doubt as to whether it functions in all people, at all times. The history of human thought, nevertheless, indicates that some individuals have shown extraordinary facility in defining the circumstances of our lives and of the world around us. The mathematicians have worked along these lines for several thousand years. No one questions their success in establishing measurements. May I remind you that they have also given us tools to establish the validity of measurements. These are conventionally referred to as "statistics." Unfortunately, the mere mention of this word releases highly emotional responses. There are those who fall into a state of paralyzed admiration whenever a group of figures is followed by the Greek letters of a statistical formula. The skeptics, on the other hand, profess to bring us back to reality with the statement that any lie can be proved by statistics.

Both attitudes are equally erroneous. Statistical analysis will give an assessment of significance when applied to properly grouped data. When applied to unrelated figures, statistics will be useless and misleading. This is equally true of simple arithmetic. One can not obtain a sum by adding apples to peaches. To the best of my knowledge this has not led anyone to suggest elimination of arithmetic from the school curriculum.

Let us now take stock of our position. New machines have given us access to hitherto unavailable information. As we have seen, the road to this information is lined with traps. At our best, we know not how to escape all of these traps. Yet, our results must be accurate to be meaningful. They must be consistently accurate. Mathematics offers tools which can monitor our performance. Why not use them?

BIBLIOGRAPHY

1. Rudolph J. Muelling: Calibration of Glassware; Instrumentation Workshop, New England Society of Pathologists, 1958.
2. Lester D. Ellerbrook: A simple Colorimetric Method for Calibration of Pipets; Amer. J. Clin. Path. 24: 868-874, 1954.
3. Joseph S. Annino: Clinical Chemistry, Little, Brown & Co., 1956, p. 10.

A NEW METHOD FOR ALKALINE PHOSPHATASE LEVEL DETERMINATION*

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With the advent of the era of psychopharmacotherapy, certain hospital-clinical-facilities have assumed added importance and have of necessity increased the work-load under which they operate. This is particularly true in psychiatric hospitals, where as a result of increasing numbers of patients receiving tranquilizing or energizing agents, and with each patient receiving a comparatively larger battery of laboratory tests before and during treatment, the demands upon the clinical laboratory have been markedly increased.

Usually the increase in laboratory personnel has not kept pace with the increased output of the laboratory; hence, any new procedure which will lessen or simplify the laboratory work-load is a desirable one. Since this experience is not confined to psychiatric institutions, it was felt that a note on a new method we have found of value might prove helpful to general hospital laboratories dealing with patients on these new drugs.

One of the common, routine tests for liver function in patients receiving pharmacotherapy is a determination of the alkaline phosphatase levels in the blood. Because of the possibility of liver damage during pharmacotherapy, this estimation is performed many, many times in each patient during the course of therapy.[1,2,3] The quantitative determination of serum alkaline phosphatase is a time-consuming one requiring the preparation of a number of reagents, the latter deteriorating in approximately one week. This makes mandatory the preparation of these reagents each five to seven days. The determination, from start to finish, requires approximately one hour, during which time the laboratory technician is pretty well occupied with the various intricacies of the test.

We have been using a modification of the alkaline phosphatase determination which involves the use of a tablet* containing buffered sodium phenolphthalein phosphate as the substrate. This is a simple, semiquantitative screening method for alkaline phosphatase levels in both jaundiced and non-jaundiced patients.

This new modification has a number of advantages. For one, all the necessary reagents for the determination are compressed into the tablet which does not deteriorate, therefore does not have to be renewed each week. An individual test can be done in 12 minutes.

It is also an extremely simple procedure requiring few manipulations. The time interval from the beginning to the end of the test may be used by the technician to good advantage in other work.

The Topeka State Hospital has approximately 600-800 patients under drug therapy, with most of them receiving alkaline phosphatase determinations on a routine basis. During the past year, the new Phosphatab determinations has been used and has been found to be satisfactory and quite simple to do.

* Received for publication February 1959.

ALKALINE PHOSPHATASE DETERMINATIONS

(500 samples)

Number of Trials	R E S U L T S 'Phosphatab'	K.A. Units*	Degree of Correlation
411	Normal	404 trials < 15	98.3%
85	Abnormal	83 trials > 15	97.6%
4	Doubtful	2 trials < 15	50%
	Doubtful	2 trials > 15	50%

* King Armstrong Units

Its reliability, by cross-check, with older methods is consistent. An occasional borderline reaction is encountered and in these instances a quantitative determination can be made. This is an infrequent occurence.

Directors of clinical laboratories who are seeking ways and means of streamlining or simplifying their laboratory procedures will find "Phosphatabs" a worthwhile addition to their armamentarium.

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* Marketed by Warner-Chilcott Laboratories as "Phosphatabs."

BIBLIOGRAPHY

1. Shay, H. and Siplet, H.: Study of Chlorpromazine Jaundice, Its Mechanism and Prevention: Special Reference to Serum Alkaline Phosphatase and Glutamic Oxalacetic Transaminase. Gastroenterology, 32:571-591, 1957.

2. Dikes, R., Achenker, V. and Deutsch, L.: Serial Liver-Function and Blood Studies in Patients Receiving Chlorpromazine. New England J. of Med., 256:1-7, 1957.

3. Hollister, L. E.: Allergic Reactions to Tranquilizing Drugs. Annals of Int. Med., 49:17, 1958.

DESIGN FOR DEVELOPMENT OF MEDICAL LABORATORIES: Personnel and Practices*

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INTRODUCTION

The purpose of this paper is to present the development, the findings, and the subsequent recommendations of a survey of existing personnel, facilities and technical practices in the medical laboratories of licensed general hospitals in Minnesota. The development of this survey is based on the primary purpose of determining effective measures that can be evolved to improve patient care services in hospitals and related institutions in the state. These measures are to be based upon an evaluation of an inventory of present practices and resources which reflect actual need.

This research and demonstration are outgrowths of earlier work made possible by the W. K. Kellogg Foundation from 1950 through 1955 which was directed toward finding ways of extending radiological and pathological services in rural Minnesota. The projects that were developed under that study were based on early deliberations of an advisory group of radiologists, pathologists and medical technologists which are summarized in the following sentences. "The . . . group felt that the most immediate need was for trained personnel in all catagories and that supplementary training for existing personnel as well as increased support for training new personnel, was needed. An intensive survey of facilities and personnel to determine the needs was considered necessary as a preliminary step."** These needs referred to x-ray and clinical laboratory personnel.

The recommended survey was conducted in 1951 by the Minnesota State Medical Association and Minnesota Hospital Association with the assistance of the Minnesota Department of Health. That study determined the number of hospitals and physicians that had laboratories and laboratory personnel, the training level of the personnel, and their interest in attending refresher training courses and utilizing laboratory consultant services. The results indicated "a need for more and better trained technicians for hospital work, . . . a real interest in visiting consultants and short courses of a week or two weeks to improve the work of existing technicians".** Consequently, five refresher courses were conducted in medical laboratory work in 1952 and 1953. They were planned on the basis of what the advisory group thought the personnel needed to know since there had been no attempt to determine what they needed to know. The success of these courses was somewhat indeterminate since there had not been an inventory of the kind and quality of work that was done by the participants before and after they took the courses.

A similar situation existed with regard to the paramedical organization recruitment activities associated with the Kellogg Foundation proj-

* Received for publication April 1959. This study was supported in part by research grant W-49 from the United States Public Health Service, National Institutes of Health, Division of Research Grants.

** From 1951 report of Kellogg Foundation Project, Minnesota Department of Health.

ect. Although it was felt that these activities were worthwhile, no measure of their effectiveness had been attempted, nor had a definitive picture of the extent or reality of the assumed shortage of personnel been concretely determined. Consequently, it was believed that staffing patterns would have to be determined on the basis of the relationships of personnel of varying training levels to the work requirements of laboratories in order to accurately appraise personnel shortage.

PROCEDURE

It is apparent from these considerations that the 1951 survey was not intensive enough to provide a sufficient inventory of practices to serve as a base line of information from which needs could be determined. Therefore, the initial objective of the medical technology program of the Hospital Services Demonstration Program undertaken in the fall of 1956 with funds provided through the USPHS grant W-49 was to obtain information about existing facilities and technical practices as well as personnel in the laboratories of licensed general hospitals in Minnesota in order to determine the measures that may be effective in improving patient care services. The survey form or questionnaire used to obtain the information was developed over a period of several months by the senior author who is a medical technologist (ASCP) serving as a medical laboratory consultant on the staff of the Hospital Services Demonstration Program. Assistance was given by representatives of the Minnesota Society of Clinical Pathologists and the Minnesota Society of Medical Technologists, and members of the Department of Biostatistics of the University of Minnesota School of Public Health. During the period of development, the questionnaire was pre-tested in eleven state-operated hospitals. The data from this pre-testing is not included in the report of findings of the survey. Six revisions of the questionnaire were necessary. The final version was approved by the representatives of the above mentioned Societies.

The questionnaire in its final form was divided into two sections. The first section contained 40 questions pertaining to personnel education qualifications, professional organization affiliations, employment policies and compensation. Accompanying this was a separate form for recording information about education qualifications, professional organization affiliations and salaries for each of the technical personnel. The second section contained 142 questions pertaining to utilization of non-technical personnel, staffing needs, laboratory test request and report practices, number and kind of laboratory tests, types of laboratory equipment, calibration of colorimetric instruments, quality control of laboratory tests, reagent preparation, training facilities and miscellaneous items.

The responses to 149 of the 182 questions in the survey form were coded and tabulated by International Business Machine equipment. There were 614 IBM cards* prepared for tabulating the first section of the questionnaire and 145** for the second section. In addition to an over-all summary of findings, two analyses were made to relate the differences found in various personnel training levels and differences found

* Since the data from the first section pertained to personnel policies, 1 card was punched for each of the personnel.

** Since the data from the second section pertained to hospital and technical practices, 1 card was punched for each of the hospitals.

in hospitals of varying size (bed capacities). These separations are defined in subsequent paragraphs.

The laboratories surveyed were limited to those in licensed general hospitals in Minnesota because earlier survey work had been done there, identifying information was available through the licensing section of the Minnesota Department of Health, and support was given by the Minnesota Hospital Association and the Minnesota State Medical Association. Between April 1957 and January 1958, a total of 181 hospital laboratories was visited by the senior author and another medical technologist (ASCP) who was also a medical laboratory consultant on the staff of the Hospital Services Demonstration Program. The data were obtained by personal interview with one member of the laboratory technical staff in all instances and with a pathologist or hospital administrator when interest was indicated. There were 183 licensed general hospitals in operation in Minnesota during this time; however, two were not visited since the bed capacities were less than six beds. Thirty-six hospital laboratories were excluded because they either did not have laboratory personnel or equipment, or they did not have the personnel to use the equipment that was in the laboratory. In all of these 36 hospitals, the laboratory work was done outside of the hospital. In addition, University of Minnesota Hospitals, Mayo Clinic, St. Mary's Hospital (Rochester), and Veterans Administration Hospital were excluded from the survey. The report of the survey includes data collected in 145 laboratories which are in hospitals of bed capacities varying from 6 to 565 beds.

The hospitals were divided into 5 groups according to bed capacity with the following resulting distribution:

GROUP	Bed Capacity	Number of Hospitals	Percent of Hospitals
A	0 — 20	18	12
B	21 — 30	37	26
C	31 — 70	48	33
D	71 — 130	18	12
E	131 and greater	24	17
Total		145	100

Generally speaking, Groups A, B, and C are found in cities of population of 6,000 and less and are designated as rural hospitals. Groups D and E are found in cities of population greater than 6,000 and are designated as urban hospitals. On the basis of this rural and urban classification, almost three-fourths of the hospitals visited are rural hospitals. This follows the general distribution of licensed general hospitals in the state.

RESULTS

The results of the survey are reported under each main topic on the basis of over-all summary analysis, personnel training level group analysis, and bed capacity group analysis. The percentage calculations were carried out to one-tenth of a percent in all cases and are reported as the nearest whole percent. Consequently, some of the totals of percents are recorded in the tables as 1% more or less than 100%.

Training Level of Personnel

There are 614 medical laboratory personnel of varying training levels included in the survey. One-third of these people are employed in the rural hospital laboratories and two-thirds of them are employed in the urban hospital laboratories. The personnel are divided into two major groups, medical technologists and laboratory assistants, on the basis of the academic and practical training they have had. The group called medical technologists is composed of technical personnel who have had academic preparation of at least two years of college including biological sciences. In addition, a large portion of them have had practical training in schools of medical technology approved by the Council on Medical Education and Hospitals of the American Medical Association. There are three training levels included in this group: medical technologist (ASCP) with a baccalaureate degree, medical technologist (ASCP) without a baccalaureate degree, and non-ASCP medical technologist, some of whom have baccalaureate degrees. The other major group called laboratory assistants is composed of technical personnel who have had one year or less training in which the academic and practical have been combined, and those who have had training on the job. There are two training levels in this group: commercial school or University of Minnesota Extension Division*†, and on-the-job or Armed Services.

Figure 1 shows that slightly more than half of the 614 personnel are medical technologists, that 14% of them work in rural hospitals and 86% of them work in urban hospitals. Nearly all of the technologists are M.T. (ASCP) and approximately three-fourths of them have baccalaureate degrees. These medical technologists (ASCP) with degrees represent slightly more than one-third of the total laboratory personnel.

Figure 1 also shows that laboratory assistants comprise somewhat less than half of all of the personnel and are almost equally distributed in rural and urban hospital laboratories. One-fourth of the 614 personnel have had commercial school training.

Twelve percent of the 614 personnel are men who are almost equally distributed between rural and urban hospital laboratories. Almost two-thirds of the men are laboratory assistants with the majority having been trained on the job or in the Armed Services. Half of the women are medical technologists (ASCP) including 36% who have baccalaureate degrees. Somewhat more than one-third of the women are laboratory assistants, the major portion having received their training in the commercial schools. Approximately two-thirds of the women work in urban hospital laboratories.

Approximately three-fourths of all laboratory personnel are employed on a regular, full-time basis with little difference existing between rural and urban laboratories. A small segment of the personnel is employed solely for emergency duty and is found almost entirely in the urban hospital laboratories.

* The University of Minnesota Extension Division offers a course for medical laboratory assistants which requires 6 months of academic and 6 months of practical work.

† A few of the personnel in this training level have completed a 12 month course for laboratory assistants at Amherst H. Wilder Dispensary, St. Paul, Minnesota.

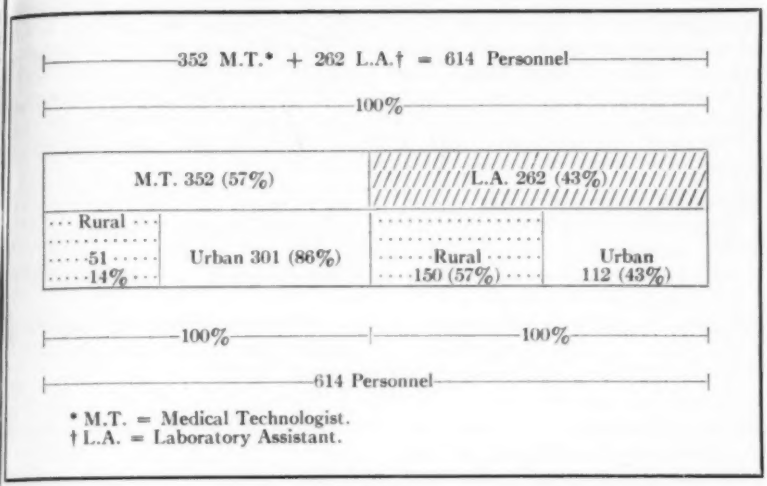

FIGURE 1
Rural and Urban Distribution of Training Level of Personnel

Duties in both x-ray and clinical laboratories are performed by approximately one-fourth of the 614 personnel. This combination of duties is found almost entirely in rural hospital laboratories. Four-fifths of the personnel performing them are laboratory assistants.

Medical Supervision of Personnel

Laboratory personnel stated that medical supervision took one of three forms: (a) no supervision from physicians, (b) supervision by a physician or committee of physicians (none of whom are pathologists), and (c) regular supervision by pathologists. Two-thirds of the 614 personnel receive supervision regularly from a pathologist with this type of direction existing almost entirely in urban hospital laboratories. In the urban hospital laboratories 92% of the personnel receive supervision from pathologists. In the rural areas, only 20% of the personnel receive supervision from pathologists with this occurring almost entirely in the 31 to 70 bed hospitals. It was found that 87% of the medical technologists are supervised regularly by pathologists and that 43% of the laboratory assistants receive such supervision. It is evident, then, that in the hospitals where there is the largest number of personnel with minimum training, there is the smallest amount of regular supervision by pathologists.

Technical Supervision of Personnel

Medical technologists act as technical supervisors* in 47% of the hospital laboratories. Thirty percent of the rural hospital laboratories receive technical supervision from medical technologists, half of whom have baccalaureate degrees. Eighty-six percent of the urban laboratories receive technical supervision from medical technologists, three-fourths of whom have baccalaureate degrees. Laboratory assistants act as technical supervisors in 52% of the hospital laboratories. Seventy percent of the rural hospitals and 10% of the urban hospitals receive technical supervision from laboratory assistants.

Personnel Fringe Benefits

The majority of personnel receive at least 9 fringe benefits:

1. A minimum of 2 weeks of vacation
2. Sick leave of 12 days per year with maximum accumulation of 12 to 24 days
3. Leave of absence granted depending upon the reason for requesting the leave
4. Terminal leave pay for accumulated time
5. 7 holidays per year
6. Social Security
7. Medical care insurance available
8. Meals at minimum cost
9. Health service facilities at the place of employment.

The regular working hours of the personnel vary from 32 to 48 hours per week with two-thirds of them working 40 hours per week. Approximately one-fifth of the 614 personnel stated that they work overtime frequently (an hour or more several days per week). Almost half of the personnel receive compensation for overtime work in the form of extra pay given to approximately one-fourth of the personnel and compensatory time to one-eighth of the personnel. Approximately one-fourth of the personnel stated that they do not work overtime at all. Five percent of the 614 personnel work alone in their respective laboratories and assume 24 hour responsibility. All are in rural hospital laboratories.

Approximately one-third of the 614 personnel work in laboratories where a separate staff is responsible for emergency duty. These people are almost entirely in the urban hospital laboratories. Approximately one-fourth of the personnel rotate day and emergency duty. This is equally common in rural and urban hospital laboratories.

Salary

Monthly salary information for the year 1957 was reported by 42[illegible] (70%) of the 614 personnel. The range of salary reported is $110 to $52[illegible] with a mean of $323 and a median of $335. These figures make no attempt to account for the value of fringe benefits or overtime or emergency duty pay. Table 1 shows that the mean and median salaries in urban hospitals are higher than those in rural hospitals.

* Technical supervision, as used here, refers to the supervision of technical details of daily laboratory procedure and testing by a medical technologist or laboratory assistant assigned to this duty in each laboratory. It has no reference to medical interpretation or diagnostic consultation which is done by the physician acting as medical supervisor of the laboratory.

TABLE 1

Mean and Median Monthly Salary of Laboratory Personnel by Hospital Bed Capacity Groups*

Bed Capacity	Person Total	Person Report	% Report	$ Mean	$ Median	$ Minimum	$ Maximum
Total	614	427	70	323	335	110	525
0-20 Beds	25	17	68	281	275	155	485
21-30 Beds	58	30	52	272	275	110	400
31-70 Beds	118	66	56	287	260	110	525
71-130 Beds	87	56	64	297	314	125	450
131 + Beds	326	258	79	346	358	200	520

* Rural hospitals: 0-20 Beds, 21-30 Beds, 31-70 Beds.
* Urban hospitals: 71-130 Beds, 131 + Beds.

Table 2 shows that the mean and median salaries for medical technologists are higher than those for laboratory assistants.

TABLE 2

Mean and Median Monthly Salary of Laboratory Personnel by Training Level Groups

Training Level	Person Total	Person Report	% Report	$ Mean	$ Median	$ Minimum	$ Maximum
Total	614	427	70	323	335	110	525
MT (ASCP) Degree	213	150	70	377	375	125	520
MT (ASCP) No Degree	76	57	75	356	357	300	525
M.T.	63	37	59	331	330	225	475
L.A.: Com. Sch. or UMED*	162	123	76	262	250	110	504
L.A.: OJT** or Armed Serv.	100	60	60	278	270	110	485

* Commercial School or University of Minnesota Extension Division.
** On the Job.

The mean salary for men is $361 and that for women is $318. It should be borne in mind, however, that the group of men reporting salary (46) is considerably smaller than the group of women (381) so that individual salary values affect the mean for men more than that for women.

Information about both monthly salary and the total amount of experience was given by 399 (65%) of the 614 personnel. Table 3 shows that there was an increase of mean salary over a period of 48 months with no trend of increase above 48 months.

Table 4 shows that the medical technologists (ASCP) with minimum experience (0-12 months) have higher mean salaries ($350 and $345) than do the laboratory assistants with maximum experience ($335 and $302).

The medical technologists (ASCP) with baccalaureate degree are the only ones who show a steady increase of salary with an increase in experience.

TABLE 3

Mean Monthly Salary of Laboratory Personnel Stating Both Monthly Salary and Monthly Experience

	MONTHS OF EXPERIENCE							
	0–12	13–24	25–36	37–48	49–60	61–120	121+	Total
Total Personnel	183	90	44	35	47	112	103	614
Reported Personnel	88	68	34	26	39	78	66	399
% Reported Personnel	48	76	77	74	83	70	64	65
Mean Salary	277	296	329	368	361	349	366	346
Minimum Salary	110	155	150	229	210	125	235	110
Maximum Salary	374	450	425	500	525	500	520	525

TABLE 4

Mean Monthly Salary of Laboratory Personnel by Training Level Groups in Monthly Experience Groups

	MONTHS OF EXPERIENCE						
Training Level	0–12	13–24	25–36	37–48	49–60	61–120	121+
MT (ASCP) Degree	$350	$357	$373	$374	$381	$381	$406
MT (ASCP) No Degree	$345	$345	$351	$368	$376	$356	$362
M.T.	$300	$360	$330	$319	$475	$354	$333
L.A.: Com. Sch. or UMED*	$235	$256	$262	$250	$283	$305	$335
L.A.: OJT** or Armed Services	$246	$250	$310	$345	$340	$312	$302

* Commercial School or University of Minnesota Extension Division.
** On The Job.

There are policies providing salary differentials for training and experience for two-thirds of the personnel. Almost all of these are in urban hospitals.

The range of total experience reported by 550 (90%) of the 614 personnel is 1 month to 564 months (47 years) with a mean of 78 months (6½ years) and a median of 48 months (4 years). Table 5 shows that there is no significant difference in mean and median experience between rural and urban hospitals.

Table 6 shows that the medical technologists have greater mean experience (90 months) than the laboratory assistants (61 months).

The mean monthly experience of women (80 months) is greater than that for men (60 months).

Organization Membership

Approximately three-fourths of the 614 personnel surveyed are eligible to be active members of organizations for medical laboratory personnel. There were three organizations taken into consideration: The

TABLE 5

Extent of Personnel Experience (Monthly) in Bed Capacity Groups*

Bed Capacity	PERSONNEL			MONTHS OF EXPERIENCE			
	Total	Report	% Report	Mean	Median	Minimum	Maximum
Total	614	550	90	078	048	001	564
0-20 Beds	25	22	88	100	072	011	372
21-30 Beds	58	55	95	088	060	001	360
31-70 Beds	118	103	87	072	036	001	480
71-130 Beds	87	79	91	075	048	001	564
131 + Beds	326	291	89	077	048	001	480

* Rural hospitals: 0-20 Beds, 21-30 Beds, 31-70 Beds.
* Urban hospitals: 71-130 Beds, 131 + Beds.

TABLE 6

Extent of Personnel Experience (Monthly) in Training Level Groups

Training Level	PERSONNEL			MONTHS OF EXPERIENCE			
	Total	Report	% Report	Mean	Median	Minimum	Maximum
Total	614	550	90	078	048	001	564
MT (ASCP) Degree	213	203	95	093	060	001	480
MT (ASCP) No Degree	76	70	92	073	054	001	348
M.T.	63	49	78	100	072	001	396
L.A.: Com. Sch. or UMED*	162	150	93	041	024	001	228
L.A.. OJT** or Armed Serv.	100	78	78	100	048	001	564

* Commercial School or University of Minnesota Extension Division.
** On The Job.

American Society of Medical Technologists, the American Medical Technologists and the National Society of Medical Technologists*. Two-thirds of those eligible for membership in one of the above three organizations can belong to the American Society of Medical Technologists. Forty-two percent of those who are eligible for this membership actually belong to the Society. One-third of the eligible personnel qualify for membership only in the American Medical Technologists or the National Society of Medical Technologists. Twenty-eight percent of those who are eligible for membership in either of these two organizations actually belong to them.

Attendance at Professional Scientific Meetings

Three main factors appear to affect the extent to which medical laboratory personnel attend professional scientific meetings: their proximity to the meeting place, their responsibilities in the laboratory, and

* An organization for medical technicians (laboratory assistants) which is similar in some respects to the American Medical Technologists. It was incorporated in 1955 in Minnesota and, reportedly, has members throughout the United States.

the expense of attending meetings. Personnel in urban hospital laboratories attend more professional scientific meetings than do those in rural hospital laboratories. Two-fifths of the personnel have attended state meetings and one-tenth of them have gone to national meetings within the previous three years.

One-tenth of the personnel stated that their work does not permit them to leave the laboratories to attend meetings. Most of these people are in rural hospital laboratories. In almost one-third of the laboratories the personnel are allowed to attend these meetings on the basis of rotation of all of the technical staff members. Most of these are in urban hospitals. The administration in slightly less than half of the hospitals assumes all of the expenses for laboratory personnel to attend professional meetings. This policy is followed by 46% of the rural hospitals and 36% of the urban hospitals.

Stated Personnel Needs

Each laboratory director was asked to state how many medical technologists and laboratory assistants are needed in addition to present staff members, basing the statement on the number of existing vacancies and upon planned expansion of laboratory services. The total stated additional need is 119 medical technologists in 56 hospital laboratories and 57 laboratory assistants in 41 hospital laboratories. The urban hospital laboratories indicated a need for 95 medical technologists and 24 laboratory assistants. The rural laboratories indicated a need for 24 medical technologists and 33 laboratory assistants. It should be noted that these stated additional needs are for 145 hospital laboratories only and do not include the number of personnel which may be required to adequately staff research, clinic, doctors' office and other medical laboratories.

It is difficult to estimate the extent to which schools educating medical technologists and laboratory assistants can satisfy the stated additional needs for these personnel because there is a lack of definitive information regarding the proportion of graduates who remain in the state, the length of time they work and other related questions. There is no doubt that efforts to interest students in the profession of medical technology should be continued since it was found that the total enrollment in all twelve of the Schools of Medical Technology* in Minnesota was 89 students which is 55% of the total capacity. In 1958, the student capacity of the course for medical laboratory assistants at the University of Minnesota Extension Division was expanded from 30 to 50 students. This course was started in 1952 at the request of the Minnesota State Medical Association, and has gained recognition to the point that each of the last two classes have had 100% enrollment, of whom approximately 90% have graduated.

Non-technical Duties in Laboratories

Non-technical personnel perform clerical and/or maintenance duties

* These are schools which are approved by the Council on Medical Education and Hospitals of the A.M.A for training students eligible to be certified by the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists.

in sixty-five of the hospital laboratories. Eighty-eight percent of the urban hospital laboratories employ this type of help and 27% of the rural hospital laboratories do so. In the laboratories where technical personnel must perform clerical and/or maintenance duties, approximately 12% of the working day is devoted to them. Most of these laboratories are in rural hospitals. In approximately three-fourths of all the hospitals the technical staff is responsible for putting laboratory test results on patients' charts and keeping records of these results for an extended period. This is done in almost all of the rural hospitals and approximately half of the urban hospitals. Approximately two-thirds of the hospitals use the system of attaching reports directly to the patients' charts (paste-on system).

Work Load in Laboratories

As was expected, there is a greater variety of procedures done in urban hospital laboratories than in rural hospital laboratories. This is believed to reflect not only differences in number of patient admissions but also the extent to which physicians request laboratory procedures for their patients. In almost all of the rural hospital laboratories the work done is limited to the following:

1. Chemical determinations of blood glucose, blood urea nitrogen, prothrombin, serum icterus index, and occasional requests for serum bilirubin, serum total protein, and total cholesterol.
2. Eight routine hematologic procedures, not including reticulocyte counts, and bone marrow biopsy studies.
3. Six qualitative and semi-quantitative procedures in urinalysis.
4. ABO grouping, Rh (anti-D) typing and crossmatching in blood banking.
5. Examination of smears and initial cultures, and occasional qualitative testing for antibiotic sensitivities in bacteriology.
6. Titration of hydrochloric acid in gastric fluids.
7. Cerebrospinal fluid cell counts and occasional determination of glucose and protein.
8. Qualitative testing for occult blood in stools.
9. Basal metabolism rates.
10. Electrocardiographs.

In the urban hospital laboratories the variety of work is extended in each area and includes serological testing, histopathology techniques, parasitology, and electroencephalography.

It was found that in all but 10 of the hospitals there are routine requests for laboratory work for patients upon their admission to the hospitals. The most common routine admission requests are for urinalysis, hemoglobin, and leukocyte counts.

All but one of the 145 hospital laboratories refer some of their work to be done outside of their own facilities. The laboratories at the Minnesota Department of Health receive specimens from 139 of the 144 hospital laboratories for serological and/or special bacteriological analysis. Approximately two-thirds of the 144 hospital laboratories refer work to hospital laboratories outside their own for a variety of procedures. A greater number and a greater percentage of rural hospital labora-

tories than urban refer work to these two places. Almost half of the 144 laboratories refer work to the University of Minnesota primarily for histopathology, special hematology and occasional chemical determinations. Although a greater number of rural hospital laboratories do this, the percentage of urban hospital laboratories doing it is somewhat larger than that of rural hospitals. Privately operated laboratories receive specimens from approximately half of the hospital laboratories primarily for chemical determinations. Here again, a greater number of rural than urban hospital laboratories refer work to these places but almost two-thirds of the urban hospital laboratories and two-fifths of the rural hospital laboratories do so.

In almost two-thirds of the hospital laboratories practically no time is spent in the preparation of reagents for all are purchased, ready for use. Eighty-one percent of the rural laboratories purchase all of their reagents ready for use, and 14% of the urban laboratories do so. About two-thirds of the laboratories who purchase some or all of their reagents use desiccated (dry-pack) reagents and almost 90% of them use fluid reagents. A larger percentage of the urban than rural laboratories use the desiccated reagents.

Laboratory Evaluation Programs

There were 122 of the 145 laboratories which indicated an interest in participating in state-wide programs for periodic survey of test results in several phases of medical technology. These are referred to as evaluation programs. Almost all of the laboratories showed a preference for surveys in hematology, chemistry and blood banking.

Tabulation of Number of Tests Done

In approximately three-fourths of the laboratories it was stated that annual reports of laboratory work are prepared. Although annual reports were requested in each laboratory, only half of them furnished copies for 1956. The systems for tabulating these annual reports varied considerably. Because of this, it is noteworthy that 94% of the laboratories indicated an interest in using a standard form for reporting work load if such were to be developed for use in the state. Because of the inconsistency of tabulation methods, the comparison of work done from one laboratory to another was made by relating the number of tests done in each major division of medical laboratory work to the total number of tests done in all divisions. For example:

$$\frac{\text{Number of Chemistry Tests}}{\text{Total Number of Laboratory Tests}} \times 100 = \% \text{ of Total Which is Chemistry}$$

$$\frac{12{,}710 \text{ (Chemistry)}}{117{,}681 \text{ (Total of all)}} \times 100 = 10.8\% \text{ of Total Which is Chemistry}$$

Table 7 shows the proportion of work load in hospitals of varying sizes that is devoted to the major divisions of laboratory work.

The percentage figures therein recorded represent the mean for each group of hospitals. As the hospital bed capacities increase, there is an increase in the proportion of work in the following fields of medical

TABLE 7

Percentage Distribution of Work Done in Laboratory Divisions in Hospitals by Bed Capacity Groups

PROCEDURES	ALL HOSPITALS			0-20 BEDS			21-30 BEDS			31-70 BEDS			71-130 BEDS			131 + BEDS		
	Total Hosp. No.	Hosp. Report No.	Work Load %	Hosp. in Group No.	Hosp. Report No.	Work Load %	Hosp. in Group No.	Hosp. Report No.	Work Load %	Hosp. in Group No.	Hosp. Report No.	Work Load %	Hosp. in Group No.	Hosp. Report No.	Work Load %	Hosp. in Group No.	Hosp. Report No.	Work Load %
Chemistry	145	83	8.5	18	6	6.7	37	9	5.3	48	34	7.5	18	14	11.1	24	20	10.5
Stools and Gastrics	145	70	0.7	18	4	0.9	37	5	0.4	48	29	0.5	18	12	0.8	24	20	0.9
Hematology	145	83	49.6	18	6	53.3	37	9	54.0	48	34	54.2	18	14	49.0	24	20	39.2
Urinalysis	145	82	19.5	18	6	25.8	37	9	23.0	48	33	18.2	18	14	18.2	24	20	18.9
Blood Bank	145	82	11.4	18	6	8.9	37	9	11.6	48	34	12.4	18	14	11.5	24	20	9.7
Bacteriology	145	74	3.0	18	4	1.2	37	8	0.9	48	29	1.1	18	13	2.0	24	20	7.4
Tissues	145	54	5.0	18	1	4.1	37	4	3.1	48	19	3.3	18	11	5.3	24	19	7.0
Electrocardiograph	145	38	2.1	18	2	3.0	37	4	3.1	48	20	2.2	18	1	1.8	24	11	2.3
Serology	145	64	3.7	18	3	3.3	37	6	2.5	48	26	3.7	18	10	3.3	24	19	4.3
Other	145	76	1.1	18	6	0.8	37	6	1.1	48	31	0.9	18	13	1.2	24	20	1.3

technology: chemistry, bacteriology, tissues (histopathology), and serology. There is a decrease in proportion in hematology, urinalysis and electrocardiography. The remaining groups are stable.

Table 8 summarizes tabulation of the mean number of admissions and mean number of tests done in hospitals by bed capacity groups.

TABLE 8

Mean Number of Admissions and Tests Done in Hospitals by Bed Capacity Groups

Bed Capacity	Total Hospitals	Hospitals Reporting Tests Done	Mean Bed Capacity*	Mean Admissions*	Mean Tests Done*	Mean Tests per Mean Admission*
Total	145	80	102	3,928	45,414	12
0-20 Beds	18	6	16	731	5,279	7
21-30 Beds	37	9	26	1,015	5,947	6
31-70 Beds	48	34	48	1,946	12,438	6
Rural	103	49	40	1,626	10,369	6
71-130 Beds	18	12	101	4,234	32,824	8
131 + Beds	24	19	263	9,670	143,745	15
Urban	42	31	200	7,566	100,808	13

* Mean figures based on number of hospitals reporting number of tests done.

On the average, in the urban hospitals there are five times as many beds, almost five times as many admissions, almost 10 times as many laboratory tests, and twice as many tests per admission than there are in the rural hospitals.

Laboratory Equipment

Urban hospital laboratories have a greater variety of equipment than do the rural hospital laboratories as might be expected from the finding that the variety of work done in urban laboratories is greater than in rural. There are 15 pieces of equipment that are found in more than 50% of all of the hospital laboratories. They are as follows:

Colorimeter	Air Incubator
Microscope	Hotplate
Centrifuge	Electrocardiograph Machine
Vacuum lines	Pipette Shaker
Bunsen Burner	Balance (Beam or Torsion)
Basal Metabolism Machine	Hot Air Oven (Drying)
Constant Temperature Water Bath	Burettes
Rh View Box	

Colorimetric Instrument Calibration

The distribution of colorimetric instruments used for chemistry determinations shown in Table 9 reveals that pre-calibrated instruments are used predominantly in rural hospital laboratories and "not pre-calibrated" instruments are used predominantly in urban hospital laboratories.

TABLE 9

Distribution of Colorimetric Instruments Used for Chemistry Determinations

Bed Capacity Groups	All Instruments	Visual	PHOTOELECTRIC		SPECTROPHOTOMETRIC	
			Pre-calib.	Not Pre-cal.	Pre-calib.	Not Pre-cal.
Total	195	5	100	11	30	49
Rural	114	3	77	2	23	9
Urban	81	2	23	9	7	40

It was possible to determine the frequency of calibration of procedures for chemistry determinations on 159 of 190 instruments (visual colorimeters excluded). Table 10 shows that regular calibration, done with each procedure, is carried out on approximately one-fourth of all the instruments.

TABLE 10

Distribution of Colorimetric Instruments Used for Chemistry Determinations Indicating Frequency of Calibration

Bed Capacity Groups	All Instruments	PRE-CALIBRATED					NOT PRE-CALIBRATED			
		Total	None	Infreq.	Occ.	E.B.*	Total	None†	Freq.	E.B.*
Total	159	109	78	12	1	18	50	16	5	29
Rural	103	92	70	9	1	12	11	6	0	5
Urban	56	17	8	3	0	6	39	10	5	24

* E.B. = Each batch of determinations.
† None = Original calibration never checked.

Although considerably more urban laboratory instruments are regularly calibrated than rural laboratory instruments, there are not as many as might be expected with the predominance of medical technologists employed in urban laboratories.

Table 11 shows that the distribution of instruments and frequency of calibration for hemoglobin determinations is almost the same, proportionately, as that for chemistry determinations.

TABLE 11

Distribution of Colorimetric Instruments Used for Hemoglobin Determination Indicating Frequency of Calibration

Bed Capacity Groups	All Instruments	Visual	PHOTOELECTRIC AND SPECTROPHOTOMETRIC									
			Pre-calibrated					Not Pre-calibrated				
			Total	None	Once	Freq.*	Wkly.	Total	None†	Once	Freq.*	Wkly.
Total	145	19	91	48	26	14	3	35	1	12	21	1
Rural	103	16	77	44	20	11	2	10	1	4	5	0
Urban	42	3	14	4	6	3	1	25	0	8	16	1

* Freq. = Checking calibration bi-annually, quarterly, monthly.
† None = Original calibration never checked.

Pre-calibrated instruments are used predominantly in rural hospital laboratories and "not pre-calibrated" instruments are used predominantly in urban hospital laboratories. Frequent or weekly calibration checks are carried out on approximately one-fourth of the instruments. Although the hemoglobin calibrations are checked frequently (or weekly) on more urban laboratory instruments than rural laboratory instruments, the higher training level of urban personnel would lead one to expect this to be done to a greater extent in urban laboratories than it is.

Information about the use of reagent blank solutions was indicated for 161 of 190 colorimetric instruments. Over half of the instruments in the rural hospital laboratories did not use a reagent blank solution. One-fifth of those in the urban hospital laboratories did not use them.

The source of solutions of standard concentration for chemical determinations was stated for 93 of the 145 laboratories. Two-thirds of these purchase all of their standard solutions from supply houses and/or the College of American Pathologists. There are 51 rural laboratories reporting the source of their standard solutions of which 43 purchase all and five purchase some of them. All 42 of the urban laboratories reported this information with 18 purchasing all and 17 purchasing some of the standard solutions.

The use of solutions of standard concentration for reference purposes (not calibration) is reported in 18 of the 145 hospitals all of which are urban.

Use of Quality Control

Four of the several accepted means of quality control[1] were selected for investigation: standard solutions for instrument calibration, reagent standardization and recovery measurement; pooled serum; and commercially prepared artificial serum (commercial control). The accuracy of information given about the use of control measures may be subject to question, for in some instances it was stated that chemical procedures were done but they were not recorded in the annual reports. In the instances where annual reports were not available, there was no way of verifying the procedures which were stated to have been done. Since 30% of the rural hospital laboratories do not keep a record of work done, cautious interpretation of these findings for rural laboratories is suggested.

Standard Solutions: Table 12 shows the number of hospital laboratories doing certain analyses and the extent to which standard solutions are used in performing the analyses.

The only chemical procedure which is done in all of the hospital laboratories is for glucose. In addition, procedures are done for the following in more than half of the hospital laboratories: urea nitrogen, cholesterol, uric acid, alkaline phosphatase, chloride and calcium. The procedure in which the largest percentage of laboratories uses a standard solution is chloride. The procedure in which the largest percentage of laboratories uses a standard solution each time a group ("batch") of determinations is made is cholesterol. It was found that 95% of the urban hospital

TABLE 12

Number and Percent of Hospital Laboratories Performing Certain Chemistry Procedures and Those Using Standard Solutions in Those Procedures

	Not Testing		Testing		OF THOSE TESTING							
					Std. Not Used		Std. Use Infrequent		Std. Use Frequent		Std. Use Each Batch	
PROCEDURE	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Glucose	0	0	145	100	64	44	18	12	31	22	32	22
Urea Nitrogen	5	5	140	95	66	48	17	12	22	16	35	25
Chloride	62	43	83	57	32	39	7	9	20	24	24	29
Cholesterol	43	30	102	70	42	41	14	14	12	12	34	33
Uric Acid	48	33	97	67	47	48	8	8	15	15	27	28
Phosphorous	93	64	52	36	16	31	9	17	12	23	15	29
Alkaline Phosphatase	60	41	85	59	45	53	9	11	13	15	18	21
Calcium	69	48	76	52	31	41	7	9	14	18	24	32
Sodium	111	77	34	23	0	0	1	3	10	29	23	68
Potassium	112	77	33	23	0	0	1	3	10	30	22	67
Creatinine	82	57	63	43	32	51	5	8	8	13	18	28

laboratories use standard solutions to varying extents for instrument calibration and reagent standardization for some or all of the procedures. Forty-eight percent of the rural hospital laboratories do so.

Recovery Measurement: Questions regarding the measurement of recovery were confined to procedures for urea nitrogen, cholesterol, uric acid, calcium, and phosphorous. All but phosphorous are done in more than half of the laboratories. It can be noted in Table 13 that the recovery measurement is used to a limited extent, varying from 2 laboratories using it in the cholesterol procedure to 21 laboratories using it in the urea nitrogen procedure.

TABLE 13

Number and Percent of Hospital Laboratories Performing Certain Chemistry Procedures and Those Using Recovery Measurement in Those Procedures

	Not Testing		Testing		OF THOSE TESTING							
					Rec. Not Used		Rec. Use Infrequent		Rec. Use Frequent		Rec. Use Each Batch	
PROCEDURE	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Urea Nitrogen	5	3	140	97	119	85	4	3	4	3	13	9
Calcium	69	48	76	52	73	96	2	3	0	0	1	1
Cholesterol	44	30	101	70	99	98	1	1	0	0	1	1
Uric Acid	49	34	96	66	88	92	3	3	1	1	4	4
Phosphorous	91	63	54	37	49	91	0	0	1	2	4	7

Standard solutions are used for the measurement of recovery of a substance in 41% of the urban hospital laboratories and 6% of the rural hospital laboratories.

Pooled Serum: Questions regarding the use of pooled serum for quality control were confined to procedures for total protein, cholesterol, uric acid, albumin, cephalin flocculation, thymol turbidity, other liver function tests (bromsulfalein, zinc turbidity, etc.), sodium, potassium, transaminase, and protein bound iodine. The first five procedures are done in more than half of the hospital laboratories. Table 14 shows that pooled serum is used to a limited extent varying from 1 laboratory using it for protein bound iodine to seven laboratories using it for total protein.

TABLE 14

Number and Percent of Hospital Laboratories Performing Certain Chemistry Procedures and Those Using Pooled Serum in Those Procedures

	Not Testing		Testing		OF THOSE TESTING							
					Pool Not Used		Pool Use Infrequent		Pool Use Frequent		Pool Use Each Batch	
PROCEDURE	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cholesterol	44	30	101	70	94	93	0	0	1	1	6	6
Uric Acid	49	34	96	66	90	94	0	0	0	0	6	6
Sodium	109	75	36	25	31	86	0	0	0	0	5	14
Potassium	110	76	35	24	30	86	0	0	0	0	5	14
Total Protein	42	29	103	71	95	92	1	1	0	0	7	7
Albumin	58	40	87	60	82	94	0	0	0	0	5	6
Transaminase	126	87	19	13	15	79	0	0	0	0	4	21
Cephalin Flocculation	66	45	79	55	75	95	0	0	0	0	4	5
Thymol Turbidity	69	48	76	52	71	93	0	0	0	0	5	7
Other Livers	106	73	39	27	36	92	0	0	0	0	3	8
Protein-Bound Iodine	141	97	4	3	3	75	0	0	0	0	1	25

It was found that 36% of the urban hospital laboratories use pooled serum for some of the procedures and 4% of the rural laboratories do so.

Commercial Control: Questions regarding the use of commercially prepared artificial serum were confined to procedures for glucose, urea nitrogen, prothrombin, total protein, chloride, calcium, phosphorous, sodium, and potassium. The first six are done in more than half of the hospital laboratories. Table 15 shows that the procedures in which the largest percentage of laboratories use a commercial control for each group of determinations is prothrombin.

In addition to the 44 laboratories using a commercial preparation, 17 use human plasma control. The commercial controls are used to a limited extent with the remaining procedures. Eighty-one percent of the urban hospital laboratories stated that commercial control is used for some or all of the procedures listed, and 75% of the rural hospital laboratories so stated.

There are eight hospital laboratories which continuously chart the values of quality control measures to determine the trend of results

TABLE 15

Number and Percent of Hospital Laboratories Performing Certain Chemistry Procedures and Those Using Commercial "Serum" in Those Procedures

	Not Testing		Testing		OF THOSE TESTING							
					Com. Not Used		Com. Infrequent		Com. Frequent		Com. Each Batch	
PROCEDURE	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Glucose	0	0	145	100	89	61	34	23	11	8	11	8
Urea Nitrogen	5	4	140	96	86	61	32	23	13	9	9	6
Chloride	61	42	84	58	43	51	24	29	8	10	9	11
Prothrombin*	9	6	136	94	60	44	6	4	9	7	44	32
Sodium	109	75	36	25	22	61	8	22	3	8	3	8
Potassium	110	76	35	24	21	60	8	23	3	9	3	9
Total Protein	42	29	103	71	55	53	28	27	7	7	13	13
Calcium	70	48	75	52	47	63	16	21	5	7	7	9
Phosphorous	93	64	52	36	33	63	11	21	4	8	4	8

* Prothrombin: 17 (13%) of the 136 laboratories use human plasma.

over long periods of time. The comparison of new reagents with old in chemistry procedures is made in 49% of all of the hospitals. This includes 79% of the urban hospitals and 37% of the rural hospitals. Sixty-five percent of all of the hospital laboratories accept commercially prepared acids and bases without question and do not titrate them against a primary standard acid before using them. Three-fourths of the 114 hospital laboratories that use buffer salt solutions accept commercially prepared buffers without checking the indicated pH. Almost half of the urban hospital laboratories follow this practice and 89% of the rural hospital laboratories do so.

CONCLUSION

The review of the survey data has resulted in proposing five programs for developing medical laboratory services in hospitals in Minnesota. Each was proposed with full realization that successful planning and implementation are dependent upon joint participation of the medical technologists, pathologists, other practicing physicians, hospital administrators and members of the staff of the Minnesota Department of Health. The programs are as follows:

1. The establishment of a laboratory which will serve as a central point from which evaluation studies may be conducted in several fields of medical technology.
2. The promulgation of consultation services in laboratory techniques and related matters in medical technology, particularly in rural areas.
3. The establishment of refresher training and specialized training courses to provide group instruction in areas of need indicated by evaluation studies and consultation.
4. The continuation of personnel recruitment programs with emphasis

on supplying more medical technologists to rural areas dependent upon recognition of this need through increased salaries and variety of work.

5. The development of a uniform system of tabulating work load in hospital laboratories to facilitate inter-laboratory comparison of work done.

Although the results and subsequent recommendations of this survey are strictly applicable only to medical laboratory services in Minnesota, it is believed that the general plan of the survey could be used in other areas of the country. Experiences of the authors confirm their belief that the method of surveying by personal interview is undoubtedly more desirable for obtaining this type of information than the more commonly employed method of mailed questionnaire. Total coverage of the hospital laboratories in Minnesota was undertaken to obtain information which would be useful in conducting the service projects resulting from this study. Should another state desire to obtain a descriptive picture of existing personnel and services but not feel the need for complete data on each of the laboratories, a random sampling study probably would provide this information at considerably reduced cost and effort. If repeated surveys are to be conducted, some details of the present questionnaire would be omitted; however all of those reported herein are considered essential for planning the development of services in medical laboratories.

SUMMARY

This is a presentation of the development, results and subsequent recommendations of a survey of existing personnel, facilities and technical practices in the medical laboratories of 145 licensed general hospitals in Minnesota. The major findings of the survey are summarized below.

1. Three-fourths of the 145 laboratories surveyed are in rural hospitals.
2. Urban hospital laboratories employ two-thirds of all the 614 technical personnel.
3. Approximately half of the personnel are medical technologists.
4. In urban laboratories most of the personnel are medical technologists. The medical supervision is given by pathologists. Technical supervision is given by medical technologists.
5. In rural hospital laboratories a large proportion of the personnel is laboratory assistants. There is almost no medical supervision. Technical supervision is given by laboratory assistants.
6. The mean and median salaries for technical personnel in urban hospitals are higher than those for technical personnel in rural hospitals. The mean and median salaries for medical technologists are higher than those for laboratory assistants.
7. Only medical technologists (ASCP) with baccalaureate degrees show a consistent increase of salary with increased experience.

8. Medical technologists have greater mean experience than do laboratory assistants.
9. Technical personnel, particularly those in rural laboratories, attend professional meetings to a limited extent.
10. The stated need for additional personnel is for twice as many medical technologists as laboratory assistants. Three times as many medical technologists are needed in urban laboratories as in rural.
11. Non-technical personnel assist with clerical and/or maintenance duties primarily in urban hospital laboratories.
12. There is a greater variety of technical procedures and equipment in urban hospital laboratories than in rural.
13. Two-thirds of the colorimetric instruments are pre-calibrated. Most of them are in rural laboratories. One-fourth of the colorimetric instruments have been calibrated each time a procedure is done.
14. Two-thirds of the laboratories purchase all of their reagents ready for use. Almost all of the rural laboratories and a small number of the urban laboratories do this.
15. Quality control measures are used to a greater extent in urban hospital laboratories than in rural hospital laboratories. Solutions of standard concentration for instrument calibration and commercially prepared artificial serum are used in more hospital laboratories than are solutions of standard concentration for recovery measurement and pooled serum.
16. Almost all of the hospital laboratories expressed an interest in state-wide evaluation studies in all phases of medical technology and particularly in hematology, chemistry and blood banking.

Five programs are proposed for developing medical laboratory services in hospitals in Minnesota.

ACKNOWLEDGMENT

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REFERENCE

1. Freier, E. F. and Rausch, V. L.: Quality control in clinical chemistry, *Am. J. of Med. Tech.* **24**: 195-207, (July-Aug.) 1958.

APPLICATION OF RADIO-ACTIVE ISOTOPES*

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The purpose of this discussion is to review briefly the application of radioactive isotopes in diagnosis and treatment. A prerequisite to the use of radioactive isotopes is certification by the Atomic Energy Commission. A license for use of isotopes is given to a physician who has fulfilled the requirements of the Atomic Energy Commission for licensure. These consist of a basic knowledge of nuclear physics and the use of isotopes in diagnosis and treatment of a certain number of patients under supervision. After fulfilling these requirements the physician may then be licensed by the Atomic Energy Commission to receive isotopes. Therefore, in order to have an isotopes laboratory it is necessary to have a physician who has the interest and inclination to obtain the training necessary for licensure. It is also necessary for the physician to have certain facilities for the handling of isotopes. These include storage facilities and instruments for detecting ionizing radiations. The equipment is readily obtainable and reasonably trouble-free. One may learn to handle the equipment after a short period of training.

The pathologist and medical technologists are well suited for handling radioactive isotopes. The department has much of the glassware needed for handling radioactive isotopes, and for the usual isotope facility only a small space is required. The pathologist has a background of training and usually sufficient scientific interest to make him a good candidate for the little additional training required for licensure. The pathologist is generally in the hospital enabling him to be aware of and available for any problems which might arise.

The medical technologist is admirably suited to handle the isotope unit as a part of clinical pathology. The background training and interest enables the technologists to rapidly learn and understand the principles involved, particularly since it is similar in certain respects to bacteriology. With radioactive isotopes however, one can detect them by using a special survey meter. A few weeks of work in a radioisotope laboratory with diligent reading of various instructions and manuals should enable most medical technologists to become proficient in handling radioisotopes.

What are radioactive isotopes and when is their use applicable? An isotope may be compared to sisters or brothers in their similarity though still having certain dissimilarities.

Each element such as carbon and chlorine, iodine, phosphorus, gold, etc. have several sister elements with the same number of protons forming the nucleus of the atom.

There is a difference in the number of neutrons which also form a part of the atomic nucleus. This then is the lack of similarity of the sister elements. This gives us the same atomic number (number of nuclear protons) but a different atomic weight for the different isotopes of an element.

So far we have only mentioned isotopes and our interest is in radioactive isotopes. By these we mean isotopes which are unstable and will

* Reprinted from The Journal of The South Carolina Society of Medical Technologists, May 1958.

undergo changes with the emission of rays or particles which will cause ionization in the medium in which they travel. Therefore these waves or particles are called ionization radiations. These ionizing radiations are the products with which we are concerned in diagnosis and treatment. The ionizing radiations with which you as medical technologists would be interested are primarily the beta particle and the gamma ray. The beta particle will travel only a short distance from the atom from which it arises, usually a few millimeters, whereas gamma rays will travel many meters.

In both diagnosis and treatment we depend upon the beta particles when using internally administered radioactive isotopes. In the use of Cobalt 60 irradiation we utilize the gamma rays.

Radioactive isotopes disintegrate at a specific and characteristic rate, such that after a certain time has elapsed, one half of the radioactivity of an element will have been dissipated. This is known as half life. There are tables which give the decay rates of radioisotopes, thus enabling us to calculate the residual amounts after a given time has elapsed. In the diagnostic use of radioisotopes we give a known amount of the substance to a patient and compare what is present at a certain time to a standard of the same amount as administered.

Of the various radioisotopes, iodine,^{131} phosphorus^{32} and gold^{198} have proved to be the most useful in the practical clinical application of isotopes.

Radioactive Iodine has an atomic number of 53, and an atomic weight of 131. It has a half life of 8.14 days and decays to stable Xenon.^{131} It is a by-product of uranium fission—eighty-five percent decays, with the emission of beta particles having a maximum energy of 0.6 mev. (average 0.2 mev.) and gamma rays with an energy of 0.364 mev. These are higher energies than are the x-rays produced by the ordinary 250 killivolt x-ray machine.

The beta particles penetrate tissue for an average of 0.6 mm.

This isotope is concentrated in the thyroid since the thyroid avidly accumulates iodine. Because of this characteristic, radioiodine^{131} has been useful in the study and treatment of certain thyroid diseases. It is used to diagnose hyper-and hypothyroidism and to treat certain cases of hyperthyroidism and thyroid carcinoma.

Radioactive Iodine is being used in several other important diagnostic tests of which clinically the determination of blood volume is the most important. This is a relatively simple procedure whereby a small quantity of iodine^{131} attached to serum albumin is given intravenously and after about 10 minutes a sample of blood is withdrawn and counted in a special type counting machine known as a well type scintillation counter. A standard is also counted and from this the blood volume may be calculated. By separating plasma and blood cells the plasma volume and/or red blood cell mass may be determined.

Another use of iodine^{131} has been through its incorporation into triolein. This is given to the patient and serial measurements of the radioactivity in the blood is made. This gives an indication of the ability of the gut to absorb fats and thereby one may differentially diagnose pancreatitis, cystic fibrosis of pancreas and other disease of the gut.

Radioactive phosphorus (P^{32}) is produced by the bombardment of

stable Phosphorus 31 or Sulphur 32 by neutrons. P^{32} has a half life of 14.3 days and on decay emits beta particles with an average energy of 0.685 mev.

Radioactive phosphorus has been used effectively in the treatment of polycythemia vera. It has also been used in patients with malignant lymphoma and metastatic diseases, however, with much less benefit than observed in polycythemia vera.

Radioactive Gold, Au^{198} is produced by neutron bombardment of elemental gold. It has a half life of 2.7 days. It decays to stable $Mercury^{199}$ and emits a beta particle with maximum energy of 0.97 mev. and gamma rays with energy of 0.411 mev. Average penetration in tissues is 1.3 mm. It is used in colloidal form within the pleural and peritoneal spaces in the presence of metastatic malignant tumors, principally carcinoma.

A reduction of symptoms has been observed in 30-50% of the patients so treated.

Short half life isotopes such as Chlorine 38 with a half life of 37.3 minutes has been used similarly as an investigative procedure.

Cobalt 60 has a half life of 5.3 years and on decay emits a beta particle with maximum energy of 0.31 mev. and two gamma rays of 1.17 and 1.33 mev. energy. Co^{60} is combined with vitamin B_{12} and used as a test in the diagnosis and differentiation of pernicious anemia from other macrocytic anemias. Urine is counted in a well type scintillation counter and by comparison with the standard counts, a value may be obtained. A low excretion (3 or 4%) indicates pernicious anemia.

There are other useful procedures employing radioisotopes and one is directed to the pertinent references for more detailed accounts of this discussion and additional procedures.

REFERENCES

1. Bierwaltes, W. H., Johnson, P. C. and Solari, A. J.: Clinical Use of Radioisotopes, W. B. Saunders Company, Philadelphia, 1957.
2. Glasstone, Samuel: Sourcebook on Atomic Energy, D. Van Notsrand Company, Inc., New York, 1950.
3. Laboratories, Abbott: Loose Leaf Manuals, North Chicago, Illinois.
4. Behrens, C. F.: Atomic Medicine, 2nd Edition.
5. Smullen, W. C., Thomas, C. C.: Basic Foundations of Isotope Technique for Technicians, 1956.

THE IN VITRO TOXIGENICITY TEST AND TYPING OF C. DIPHTHERIAE UNDER EPIDEMIC CONDITIONS*

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Introduction

The bacteriological diagnosis of diphtheria since its inception at the beginning of the present century has been concerned with the rapid and accurate identification of virulent organisms from patients and carriers. Essentially, it has consisted of the morphological identification of the organism on Loeffler's medium and the demonstration of toxigenicity by animal inoculation. Under panic conditions of an outbreak many laboratories would be handicapped by lack of animals for the performance of virulence tests. The modified Elek *in vitro* test for the toxigenicity of *Corynebacterium diphtheriae* (King et. al. 1949; King et. al. 1950) has been used routinely at the Communicable Disease Center Laboratory, Chamblee, Georgia for a number of years. This technic was used with satisfactory results by the Detroit Department of Health Laboratory during a diphtheria outbreak at Detroit, Michigan in 1956. Virulence tests in animals were also performed during this period hence the efficiency of an *in vivo* method and of the *in vitro* test is compared under the existing epidemic conditions. At that time experience was also gained concerning the typing of *C. diphtheriae* which furnished information of value for the health department epidemiologist.

Methods

Cultures

The *C. diphtheriae* cultures used in this study were isolated in November and December of 1956 during an outbreak in Detroit, Michigan. Because of the large volume of work involved, Loeffler's cultures and guinea pig virulence tests were done by one laboratory department while another performed the *in vitro* virulence plates and cultured nose and throat swabs from patients in Herman Kiefer Hospital. The nose and throat swabs were immersed in 0.5 ml. tryptose broth pH 8.0 and delivered to the laboratory as soon as possible where they were streaked on Kellog and Wende tellurite plates. At 24 and 48 hrs., all suspicious colonies were transplanted to Loeffler's slants from which presumptive morphological reports were rendered and virulence plates inoculated.

Virulence Tests

The modified Elek *in vitro* toxigenicity test was performed as described by King and co-workers (1949-1950). The M-4 base is prepared as follows: proteose peptone (Difco), 2.00 gm.; granulated agar (Difco), 1.75 gm.; sodium chloride (C.P.), 0.25 gm., distilled water, 100 ml., pH adjusted to 7.8; autoclave 15 minutes at 15 lbs.; dispense in 10 ml. quantities and store at room temperature. Not all proteose peptones are satisfactory, a fact known to Elek and reported upon by Herman et. al. (1958). Each lot of proteose peptone must be checked with known toxigenic strains of *C. diphtheriae*. To prepare a plate put 1.5 ml. of 0.3 per cent potassium tellurite (Parsons et. al. 1955) and 2.0 ml. of specially prepared rabbit

* Received for publication February 1959, reprinted by permission from Michigan Bulletin

serum (King et. al. 1950) in a sterile abrasion-free petri dish. Pour 10 ml. of melted base into the plate and mix thoroughly. Filter paper strips impregnated with diphtheria antitoxin (Wyeth diluted to 500 units per ml.) are pressed into the agar before hardening. After the surface of the plate is dried at 37° C., inoculation is made across the plate at right angles to the paper strip. A toxigenic *minimus* strain should be used on each plate as a control. A positive result consists of grey precipitation lines in the agar beginning at the inoculum and extending outward at 45 degrees from the filter paper strip. Lines appear after 24 to 72 hours of incubation at 37° C.

The *in vivo* virulence method used was the subcutaneous inoculation of two guinea pigs with 1 ml. of a pure broth culture of the test organism. The control pig received 500 units of diphtheria antitoxin intraperitoneally two to four hours before injection of the broth suspension. If the culture was virulent, the test animal usually died on the second or third day with hemorrhagic adrenal glands revealed upon autopsy while the control pig remained healthy. Guinea pigs were observed for five days before the cultures were reported as avirulent.

Typing

C. diphtheriae cultures were typed following the procedure outlined by the Communicable Disease Center. Heart infusion broth pH 7.8 was inoculated with pure cultures of the test organisms, incubated at 37° C. for 24 hr. and used as the inoculum for the following: heart infusion broth, dextrose, saccharose, starch and glycogen broth and McLeod chocolate-tellurite plates. Bacto purple base adjusted to pH 7.8 was used for the fermentation tests and all cultures were incubated at 37° C. The carbohydrate fermentations were recorded daily for seven days. Heart infusion broth cultures were observed for pellicle formation. After 48 hrs.' incubation, these cultures were used for the hemolysin test with pooled human cells. Colony characteristics were observed on McLeod plates after 48 hrs.' incubation.

Results

C. diphtheriae was isolated from 36 patients at Herman Kiefer Hospital by both laboratory departments. These cultures were isolated from separate clinical specimens taken at approximately the same time. Each department used its own cultures for the virulence tests; one using

TABLE 1
In Vivo and _In Vitro_ Toxigenicity Tests With 102 Cultures of _C. diphtheriae_

Virulence Test	Cultures from a Series of 36 Patients*	Cultures from a Series of 66 Patients†
Negative by both tests	11	27
Positive by both tests	20	28
Positive by **in vivo** only	1	5
Positive by **in vitro** only	4	6
Total Positive **in vivo** tests	21	33
Total Positive **in vitro** tests	24	34

* The virulence tests were performed with separate cultures from duplicate clinical specimens taken at approximately the same time.

† These cultures were tested by the **in vivo** method upon isolation and then stored for approximately three months before examination by the **in vitro** test.

animal inoculation, the other the *in vitro* plates. Sixty-six *C. diphtheriae* cultures that had been tested for virulence in guinea pigs and then stored were later examined by the *in vitro* method. The result of these two groups of cultures are presented in Table 1. Thirty-one of the 36 cultures or 86% were in agreement. Of the 4 cultures which were avirulent in guinea pigs but virulent on the *in vitro* plate, 3, were *minimus* strains. According to Herman et. al. (1958) *minimus* strains are prone to produce small amounts of toxin which may not be detected by animal methods. In the series of 66 cultures *in vivo* and *in vitro* virulence results were the same for 55 cultures or 83%. There were 5 *minimus* strains among the 6 cultures which were avirulent in guinea pigs but subsequently proved to be virulent by the *in vitro* method. Some of the discrepancies that occurred in these two groups of cultures may have been due to the loss of a virulent strain in mixed cultures during replating since the small *minimus* colonies are more difficult to recognize than the larger colonies of the other strains.

Eighty-five strains of *C. diphtheriae* isolated from 84 patients at Herman Kiefer Hospital were typed and their virulence determined by the *in vitro* method. The results which are indicated in Table 2 were confirmed by Dr. E. I. Parsons and associates at the Communicable Disease Center Laboratory, Chamblee, Georgia.

TABLE 2
In Vitro* Toxigenicity Tests and Types of 85 Strains of *C. diphtheriae

Type	Number of Strains Tested	Virulent Strains	Avirulent Strains
Gravis	1	1	0
Mitis*	39	19	20
Intermedius	1	0	1
Minimus*	44	44	0
Total	85	64	21

* One clinical specimen yielded a mixed culture of a virulent **minimus** strain and an avirulent **mitis** strain.

Approximately one-half of these cultures were *minimus* strains, all of which proved to be toxigenic. Most of the other cultures were *mitis* strains but only half of these were virulent. As indicated, one clinical specimen contained both an avirulent *mitis* strain and a virulent *minimus* strain. With such cultures it is quite possible to miss one of the strains while working under the pressures of epidemic conditions.

Discussion

A number of difficulties were encountered during the initial attempts to perform the *in vitro* test. These included the procurement of a satisfactory lot of proteose peptone and the necessity that the pH be at least 7.8 for consistently reliable results. Also it was necessary to exercise great care in the collection of serum so that no hemolysis occurred. Recently, Herman et. al. (1958) reported the preparation of a satisfactory substitute for serum which eliminates such problems. This "additive" is prepared from glycerine, Tween 80, and Bacto-Casamino acids and is used in the plates in the same quantity as the serum for which it is substituted. After approximately 100 comparative tests showing

consistently good results, the "additive" has been used routinely in our laboratory for the past year. Definite lines of precipitation may appear a few hours later than with rabbit serum. Both the additive and the modified base (M-4) are now commercially available (Difco Laboratories).

Summary

1. One hundred and two *C. diphtheriae* cultures isolated during an outbreak were examined for toxin production by the subcutaneous injection of guinea pigs and the modified Elek *in vitro* test. Eighty-four per cent of the cultures gave the same result by both methods while 6% were virulent only by the guinea pig method and 10% only by the *in vitro* test.

2. Eighty-five strains of *C. diphtheriae* were typed and tested for toxigenicity by the *in vitro* method. Approximately one-half of these cultures were *minimus* strains and the remainder were chiefly *mitis* strains. All *minimus* strains were virulent while *mitis* strains were variable in this respect.

3. The data presented in this report indicates that the *in vitro* test is capable of giving results as good or slightly better than the subcutaneous guinea pig method under epidemic conditions. After a little experience the technic was found to be simple, time-saving and inexpensive. With commercially available materials the *in vitro* toxigenicity test can be seriously considered for use in the small hospital laboratory.

Acknowledgments

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* Deceased

REFERENCES

1. Herman, G. J., Moore, M. S., and Parsons, E. I.: A substitute for serum in the diphtheria *in vitro* toxigenicity test. Tech. Bull. Reg. M. Technol., **28:** 11-13, 1958.
2. King, E. O., Frobisher, M. Jr., and Parsons, E. I.: The *in vitro* test for virulence of *C. diphtheriae*. Am. J. Pub. Health, **39:** 1314-1320, 1949.
3. King, E. O., Frobisher, M. Jr., and Parsons, E. I.: Further studies on the *in vitro* test for virulence of *C. diphtheriae*. Am. J. Pub. Health, **40:** 704-707, 1950.
4. Parsons, E. I., Frobisher, M., Moore, M., and Aiken, M. A.: Rapid virulence test in diagnosis of diphtheria. Proc. Soc. Exper. Biol. & Med., **88:** 368-370, 1955.

A SIMPLE DEVICE FOR CALIBRATING TEST TUBES*

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In the clinical laboratory, many routine procedures can be performed using "home calibrated" test tubes. The authors have designed a simple device which may be used to mark calibrations rapidly and accurately on test tubes or other cylindrical glassware. The use of this device has resulted in considerable savings on costs of items having high breakage rates.

The materials used in the construction of this device were easily obtainable. Three components were purchased—the rest were scrap pieces of wood and metal. The purchased items were a diamond-point pencil,[1] a hosecock clamp,[2] and a dovetail support clamp and socket.[3] Total cost of these items was $5.00.

To calibrate a tube using the device, fill the test tube with the desired amount of water from a burette or volumetric pipet. Insert the tube in

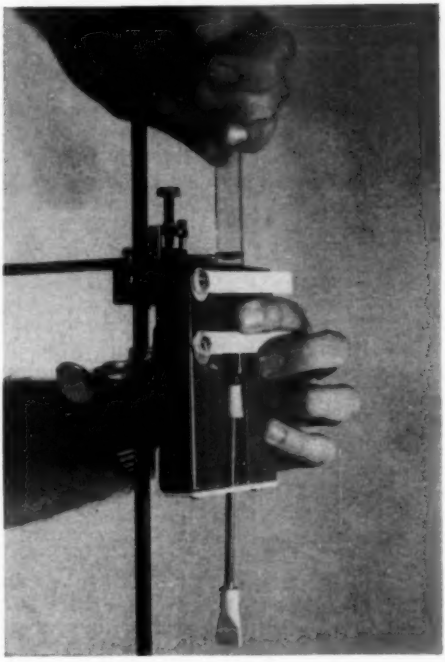

FIGURE 1

* Received for publication January, 1959.

the groove behind the spring. (Fig. 1). Using the adjusting screw, bring the bottom of the meniscus even with the pencil point. Then carefully rotate the tube against the point to produce a smooth circle around the tube.

Summary

A simple homemade device is illustrated for use in calibrating glassware. Instructions for calibration of glassware are given.

REFERENCES

1. A. S. Aloe Co., Minneapolis, Minn., Catalog No. 64830.
2. Ibid; Catalog No. 29640.
3. Ibid; Catalog No. 29500 & 29504.

THE LABORATORY DETECTION OF CARBON MONOXIDE POISONING*

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Introduction

In modern life there are several sources of poisoning with carbon monoxide gas less easily recognized than the automobile exhaust pipe. We should be aware of the carbon monoxide hazard in the use of stoves burning petroleum fuels when such stoves are operating improperly or when the oxygen available for combustion is inadequate in amount. Carbon monoxide will often be formed when a substance containing carbon is burned in a very limited amount of oxygen. Use of a charcoal burner in a closed room provides the suitable conditions. It has been reported that the blood of people who are heavy smokers may contain as much as five per cent carbon monoxide hemoglobin (HbCO).

In the state of Vermont each winter there are several deaths attributed to the accidental inhalation of carbon monoxide. In addition, an unrecorded number of people collapse but recover after being removed to a pure atmosphere if respiration is maintained.

The laboratory may be called upon to state whether a sample of blood obtained from such a person contains a significant amount of HbCO. In such instances, the presence of HbCO in high concentrations may be demonstrated by simple chemical tests based upon the facts that normal blood samples have their oxygenated hemoglobin (HbO2) converted to brown pigments when heated or allowed to react with strongly alkaline solutions whereas HbCO remains reddish. Several simple tests of this type have been described (Gradwohl, 1956) (Wintrobe, 1956).

Methods

The simplest test of all requires no reagents. Simply boil a part of the suspected blood and a normal control sample in test tubes for a few minutes. The normal control becomes brownish. A blood sample containing a large amount of HbCO remains red. The boiled samples may be compared with the untreated samples. This test may not detect HbCO unless present to the extent of 30% or more of the total hemoglobin.

Wintrobe has described a simple spot test which has about the same sensitivity. Normal blood and the suspected blood are placed side by side on a spot plate and an equal volume of 25% sodium hydroxide solution added. HbO2 turns brownish; HbCO does not.

A simple, semi-quantitative test (Yant and Sayers, 1923) will detect concentrations of HbCO in excess of 25% of the total hemoglobin. Diluted blood is mixed with a freshly prepared tannic acid reagent. Visual comparison is made with similarly-treated standards made from diluted normal blood saturated with carbon monoxide and mixed in

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suitable proportions with diluted normal blood not containing carbon monoxide.

Use of a microspectroscope is a more delicate and dependable method for the detection of HbCO when the concentration is low. Two different types of spectroscopes are available, the grating spectroscope and the prism spectroscope, which differ only in the optical systems. In the former type, the spectrum is produced by diffraction of the incident light with formation of a spectrum which is uniform throughout; that is, there is no compression of the bands in the regions representing the longer wavelengths nor expansion in the region of the shorter wavelengths, as in the case with a prism spectroscope. This phenomenon is due to changes in the index of refraction of the prism at various wavelengths. These differences make the calibration of a prism spectroscope more time-consuming.

Many methods are available for use in calibration, including use of the flame spectra of ions of sodium, lithium or potassium which have clear lines, widely spaced. These may be elicited from a flame photometer.

For the calibration of a grating spectroscope, the use of two or three lines from such emission spectra and reference to a table of wavelengths of the principal lines will complete the task. In the case of a prism spectroscope the calibration curve, in a system of Cartesian coordinates, is non-linear; thus a calibration curve having many points must be constructed. Toward this end, the use of a helium discharge tube is quite convenient since thirteen useful lines may be observed. The thirteen-point curve constructed by using the wavelength values (Hodgman, 1951) of these lines is then made the basis for a table of wavelengths corresponding to scale readings.

The validity of the curve may be established by independent observations, such as the mercury line at 546 millimicrons from a fluorescent lamp and the neon line at 585 millimicrons from an incubator indicator lamp. Then the absorption spectra of HbCO and HbO2 may be studied by placing diluted samples in cuvettes with plane sides and directing a source of white light toward the slit of the spectroscope through the interposed cuvette.

Studies of hemoglobin derivatives in pure form are quite simple. Directions for making such derivatives have been reported (Sunderman, 1953). The shift to clinical material impresses the observer with the fact that as the concentration of HbCO decreases its detection is more difficult. This is partly due to its association with HbO2. The latter compound absorbs strongly and is almost invariably present at some stage of testing. Differentiation of the two compounds is accomplished by making use of the fact that the two absorption bands of HbO2, from 535 to 550 and from 571 to 584 millimicrons, disappear on addition of a few crystals of sodium hydrosulfite (dithionite) whereas the bands of HbCO in the same region are not similarly affected. Thus if such bands are not altered by this reagent, HbCO is present. With this technique a good spectroscope will permit detection of as little as 6% HbCO.

After such studies as the above have been made, various quantitative methods (Consolazio, 1951) (Drabkin, 1950) may be employed, if warranted.

Discussion

The chemistry of carbon monoxide poisoning is, first and foremost, the chemistry of the combination of carbon monoxide gas with the respiratory pigment, hemoglobin. Oxygen uptake in the lungs is inhibited by the presence of carbon monoxide in relatively low concentrations. The insidious effect of low concentrations of carbon monoxide in air which is breathed is evident from the observation (Barcroft, 1928) that hemoglobin combines completely with carbon monoxide at 0.16 mm. of mercury pressure or with oxygen at 38 mm. of mercury pressure. HbCO in an individual's blood must reach rather high concentrations, generally of the order of 50 per cent or more of the total hemoglobin in non-anemic persons, in order to cause death.

A simplified view of the hemoglobin molecule shows four heme (ferrous protoporphyrin) molecules joined with one globin molecule to make up the whole. The apparently identical manner in which both oxygen and carbon monoxide become ligated to the ferrous atoms of the heme molecule (Pauling, 1949) indicates that combination with either gas takes place only at the expense of reduced combination with the other. This leads to the concept that anoxia is due to the greater avidity of carbon monoxide rather than oxygen for the ferrous atoms.

Pauling's studies of the magnetic properties of hemoglobin contribute to an explanation of the kinetics of the combination. His studies indicate that the four nitrogen atoms in the porphyrin ring system form a square, in the center of which there is located a ferrous atom which is electrically neutral because of the formation of four bonds with the nitrogen atoms. If the postulate of the approximate electrical neutrality of all atoms in stable compounds is employed, it can be reasoned that carbon monoxide and oxygen can combine with the heme group without upsetting the energy state of the iron atom because of their ability to form covalent bonds. It is believed that this occurs and that it accounts in part for the easy gain or loss of these gas molecules by the hemoglobin molecule in vivo. But our ideas of valence are apparently still too imperfect to do more than suggest the probable nature of the bonds which are formed since carbon monoxide appears to be a resonance hybrid involving three structures so that the iron-carbon bond also resonates (Wyman, 1948).

Other evidence of the identity of the linkage mechanisms of the two gases, such as magnetic changes and equilibrium curves (Wyman, 1948), suggests why the absorption spectra of HbCO and HbO_2 are almost indistinguishable.

The recent development of synthetic oxygen-carrying compounds, thus far restricted to chelates of cobalt, promises to be of considerable help in the investigation of problems of this type since the synthetic compounds are readily investigated by physical and chemical methods, whereas the study of the natural oxygen carrier, hemoglobin, is made difficult by its protein nature.

Summary

Elements of the physical chemistry of carbon monoxide poisoning are discussed.

Simple methods, chemical and spectroscopic, are presented for the detection of carboxyhemoglobin.

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REFERENCES

1. Barcroft, J., The Respiratory Function of the Blood. Part II, Hemoglobin, Cambridge University Press, London, 1928, 200 pages.
2. Consolazio, C., Johnson, R., and Marek, E., Metabolic Methods, C. V. Mosby Co., St. Louis, 1951, page 285.
3. Drabkin, D., (in) Medical Physics, Vol. II, Glasser, O. (ed.), The Year Book publishers, Chicago, 1950, page 1076.
4. Gradwohl, R., Clinical Laboratory Methods and Diagnosis, C. V. Mosby Co., St. Louis, 1956, page 2352.
5. Hodgman, C. D., Handbook of Chemistry and Physics, Chemical Rubber Publishing Co., Cleveland, 1951, 2894 pages.
6. Pauling, L., The Electronic Structure of Hemoglobin (in) Roughton, F., and Kendrew, J., (eds.), Haemoglobin, Interscience Publishers, New York, 1949, 317 pages.
7. Sunderman, F. W., et al., Am. J. Clin. Path. 23:575, 1953.
8. Wintrobe, M. M., Clinical Hematology, Lea and Febiger, Philadelphia, 1956, page 389
9. Wyman, J., Heme Proteins (in) Advances in Protein Chemistry, Vol. IV, Academic Press, Inc., New York, 1948, 575 pages.
10. Yant, W., and Sayers, R. R., Carbon monoxide determination in the blood, Bureau of Mines Technical Paper 373, 1923.

DETERMINATION OF FECAL LIPIDS*

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Introduction

Although the fractional determination of fecal lipids is of considerable value in the differential diagnosis of some diseases, the performance is discouraged by some laboratories because of the disagreeable nature and time involvement of such procedures. The usual method of extraction by solvent layering and separation are in general disliked by technologists and technicians. Also the point of heat drying has been brought up by many as adding to inaccuracies because of the breakdown of lipids and the volatility of their fatty acids. Therefore, the following method is suggested because of its great reduction in time, lack of heat and increase in reproducible results:

Material

Material other than the usual laboratory equipment (beakers, stirring rods, scales, etc.) is a Standard Soxhlet apparatus with Allihn 50 mm

Figure I

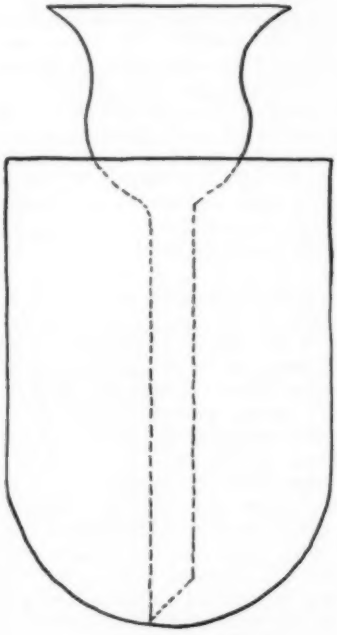

Figure II
Thimble and Beveled Thistle Tube

*Presented before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June 1958.

condenser (Fig. 1) with fat free paper thimbles of single weight 100 x 40 m.m. (Fig. 1 and 2) electric fan and thistle tube (Fig. 2).

Reagents

(1) Concentrated HCl. Reagent grade.
(2 Petroleum Ether (B.P. 30°-60° C). Reagent grade. Must have no titratable acid when titrated with 0.1 Normal sodium ethylate & phenolphthalein as an indicator.
(3) 1.0% alcoholic phenolphthalein.
(4) 0.1 Normal sodium ethylate. In a liter volumetric flask place 700 cc (about) absolute ethyl alcohol and 2.3 gm. freshly cut metallic sodium. When dissolved dilute to volume with alcohol, titrate with standardized 0.1 Normal HCl with 1.0% alcoholic phenolphthalein as an indicator and adjust to 0.1 Normal if necessary.

Procedure

(a) Approximately 5 gm, of feces (more or less depending on consistency) are roughly weighed on a balance in a 50 ml beaker. Grinding is seldom necessary, however, if feces is granular, it can be ground in a mortar. If excess mucus is present add a little (about ⅛ to ¼ gm) of papain. Sufficient distilled water is added to make a mixture that can be poured. The more viscous this mixture the better. To this add 0.1 cc concentrated HCl to each cc total volume. (Example: you use 5 gm formed feces to which is added 10 cc water, the total volume being about 15 cc. Then 1.5 cc HCl is added). Allow to stand for 20 to 30 minutes to convert all soaps to fatty acids.

(b) Label a paper thimble single weight fat free with patient's name in pencil. Weigh on analytical balance to first decimal place and record weight on thimble with pencil. Now weigh to second decimal place and record last decimal place on thimble.

(c) Pour the acidified fecal suspension into the thimble tilt while rotating the thimble so as to form a very thin layer upon the wall of the thimble, extending from ½ to ¾ of the way up the tube (Fig. 3). The thimble is continually rotated until the thimble has absorbed most of the water of the sample. This rotation must be constant so as to cause an even distribution of the sample upon the inside of the tube. When the absorption is complete, place in front of an electric fan to dry overnight. **DO NOT PLACE IN OVEN TO DRY.**

(d) The next morning the dried thimble with the thin layered sample is weighed. At this point it is well to note there is almost no objectionable odor. The difference in the weight of the thimble and of the dried sample and thimble should be recorded in milligrams.

(e) The dried sample in the thimble is placed in the Soxhlet apparatus wet down from the top of the thimble with petroleum ether (B.P. 30°-60° C., reagent grade, free from titratable acid) so the fat will not be carried above the solvent level by capillary action. Now the thistle tube with the beveled end is placed to the bottom of the thimble. The Thistle Tube is necessary to obtain complete extraction since the layer of the sample diminishes porosity of the thimble wall. The Thistle Tube will cause circulation of the solvent continually (Fig. 2). The allihn condenser is put in place. About 150 cc

of petroleum ether is introduced through the condenser. Now extract for 2 hours with the petroleum ether.

(f) At the completion of the extraction time remove the flask and add 4 drops of alcoholic phenolphthalein indicator (1.0%). Titrate while hot with 0.1 normal sodium ethylate. Each 1 cc of sodium ethylate is equivalent to 28.3 mg of fatty acid. The percent fatty acid is calculated from dry weight obtained above.

$$\frac{\text{cc of sodium ethylate} \times 28.3}{\text{dry weight of specimen (mg)}} \times 100 = \text{fatty acid \%}$$

(g) Dry the extracted thimble by placing in front of electrical fan for 4 hours or until constant weight is achieved. The difference from the pre-extraction dry weight gives weight of total fat. Total fat minus the fatty acid is equal to neutral fat.

Results

By this method the normal values are close to the normal values obtained by the more laborious wet method and are sufficiently accurate for clinical purposes. The following table shows the results of four samples run by each method. These methods show only a small variation of about 1%. Recovery of added fats and fatty acids are very good.

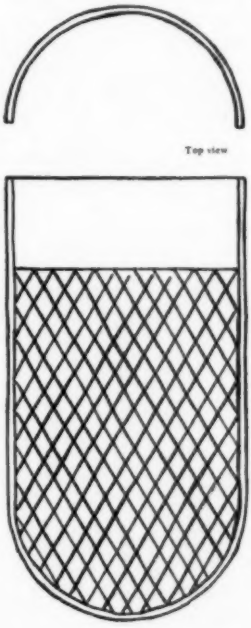

Figure III
Side View Showing Cross Section of Thimble and Sample Inside of Thimble

Results of four samples run by dry (our method) and wet method. All results given in percent.

SAMPLES	% Free Fatty Acids		% Neutral Fats		% Total Fats	
	Dry	Wet	Dry	Wet	Dry	Wet
1	2.1	1.1	35.1	33.2	37.2	34.6
2	4.1	3.6	19.2	18.8	23.3	22.5
3	12.2	11.1	4.8	6.3	17.1	17.4
4	11.1	9.2	13.1	14.1	24.1	22.3

Discussion

The chief objection to the use of dried stool samples is based on the fact that many stools are alkaline and in the presence of heat the fats are hydrolized to fatty acid giving results not representing the true proportion of these substances in the original specimen. By producing a thin layer on the absorbent thimble drying can be accomplished in an acidified stool without the use of heat thereby eliminating the chief objection to the use of dried specimens. The objection of the personnel is also overcome in that the here-to-fore bad odor involved in running stool fats is eliminated almost completely. Also the reduction in time makes it now a practical laboratory test, thereby overcoming the feeling that it is expensive and technically demanding. We do not feel that any information of great clinical value is obtained by a separate determination of soaps. In this procedure they are converted to fatty acids by the action of the HCl and are included in the fatty acid fraction.

The values obtained by this method are all reported as percent of dry weight. Normal values being equal to those obtained by the wet extraction method.

Normal total fat not more than 27%.
Normal neutral fat not more than 12%.
Normal fatty acids not more than 16%.

Elevations in neutral fats are interpreted to indicate improper fat digestion (i.e.: pancreatic deficiency, diarrhea).

Discussion Continued

Elevations in fatty acid are interpreted to represent a defect in absorption (i.e.: Obstructive jaundice, steatorrhea, diarrhea, etc.).

We feel that this procedure will prove of great value in diagnosis of certain conditions, and that the great saving in time will be of great benefit to the clinical laboratory.

Objective

The objective of this work is to find a more efficient and effective way to perform the quantitative determination of fecal fat contents.

Acknowledgment

It is with sincere thanks and appreciation that I wish to acknowledge Dr. Shelley Swift to whom most of the credit is due. To Josephine Rust Goodridge and the laboratory staff of St. Marks Hospital goes much thanks. To Doris Rust for her help in the clerical field. To Cleora A. Webb, for help and encouragement; to all these my thanks.

References

1. Fowlweather, F. S., and Anderson, W. N.—Journal of Biochemistry, Volume 40, pages 350-351, 1946.
2. Fowlweather, F. S.—British Journal of Experimental Pathology, pages 7-14, 1926.
3. Ham, Thomas Hale—A Syllabus of Laboratory Examinations in Clinical Diagnosis, First Edition, Fifth Printing, Cambridge, Massachusetts. Pub. Harvard University Press—1955.
4. Hepler, Opal E.—Manual of Clinical Laboratory Methods. Fourth Edition. Seventh Printing, Chicago, Illinois. Pub. Charles C. Thomas—1956.

A CASE OF HEMOLYTIC DISEASE OF THE NEWBORN DUE TO PURE ANTI-Kp^b (Anti-Rautenberg)*

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Here presented are two cases of Anti-Rautenberg which are illustrative of newer knowledge of the Kell system. One case came to light as the result of a hemolytic transfusion reaction; the other through hemolytic disease of the newborn. To the best of the authors' knowledge, Rautenberg has not been associated with hemolytic disease of the newborn.

From 1946 when Kell was discovered by Coombs, Mourant and Race;[3] followed by Levine, Backer, Wigod and Ponder's discovery of Cellano three years later,[5] to 1956, the Kell system appeared to be a simple straightforward two allele system. In 1956 however, Allen, Lewis and Fudenberg[1,2] established by a series of brilliant investigations that the Kell system involves at least two additional antigens, Penney and Rautenberg. Penney and Rautenberg are considered to be alleles related to Kell and Cellano just as E and e in the Rh system are related to C and c.

The antigen Penney occurs infrequently, being present in only about two per one hundred of the population. Rautenberg occurs more universally and Allen reports only 2 bloods negative in 5500 tested.

The case of Mrs. A. B., involving hemolytic disease of the newborn illustrates this new relationship clearly. (See Figure No. 1.)

Shown are the reactions obtained when the mother (the propositus of this case), her husband and their baby are tested with the 4 antisera involved in the Kell system.

All three are negative with Anti-Kell serum, and positive with Anti-Cellano as expected. This agrees with the understanding we have all had regarding the Kell system. That is, if a blood types negative for one of a pair of alleles, it may be expected to be positive for the other.

Consider now the reactions obtained with Anti-Penney and Anti-Rautenberg sera. These too are alleles, just as E and e in the Rh system. A blood may be positive for both, as the baby is in this case; or may be negative for either one and therefore positive for the other, as is the mother and the father.

	(k) ANTI-KELL	(k) ANTI-CELLANO	(Kp^a) ANTI-PENNEY	(Kp^b) ANTI-RAUTENBERG
MOTHER (PROPOSITUS)	0	+ (WEAK)	+	0
FATHER	0	+	0	+
BABY	0	+	+	+

Figure 1
Case of Mrs. A. B.

* Received for publication Feb. 1959. Read before the 11th Annual Meeting of the American Association of Blood Banks, Cincinnati, Ohio, November, 1958.

Allen and his co-workers have proposed a system of gene combinations on the chromosomes which aids in the understanding of the inheritance of these factors and their relationship to one another. (See Figure No. 2.)

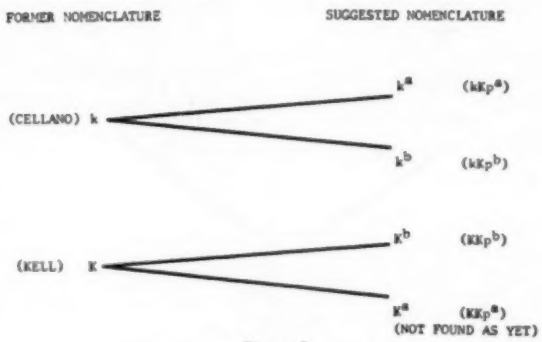

Figure 2
(After Allen, Lewis and Fundenberg—1958)
K^o NO K, k, Kp^a, Kp^b
(After Chown, Lewis and Kaita—1957)

Allen has suggested that the gene called k or Cellano, be considered either of two genes: namely k^a, which is a combination of Cellano plus Penney, or k^b, which is a combination of Cellano plus Rautenberg. The gene k^a, usually distinguishes itself by weak reactions with Anti-Cellano serum.

The gene known as K or Kell should also be considered as either of two genes, K^b, which is Kell plus Rautenberg, and K^a, which is Kell plus Penney. This latter gene has not as yet been found, and is believed to be very rare, if existent at all.

In addition Chown, Lewis and Kaita have reported a family in which two members presented no known antigens of the Kell system.[4] That is, no Kell, Cellano, Penney or Rautenberg. They have suggested the name K^o for the gene responsible for this. Just where and how this may fit into the Kell picture is not yet clear.

Applying this inheritance scheme to the individuals in the case of Mrs. A. B., we get a clearer understanding of what is happening. (See Figure No. 3.)

The mother being negative for Kell has its allelle Cellano present on each chromosome. She is also negative for Rautenberg and has its allele Penney present on each chromosome. She then, is homozygous for Cellano and Penney or to use the shorthand terminology a k^ak^a.

The father has the same double dose of Cellano, but is negative for Penney and therefore has Rautenberg on each chromosome. He is homozygous for Cellano and Rautenberg, or a k^bk^b.

The baby also has a double dose of Cellano, and as expected, is positive for each of the alleles, Penney and Rautenberg. He is a k^ak^b.

The original Anti-Rautenberg serum came from a man who had received a large number of transfusions, which were responsible for his immunization.

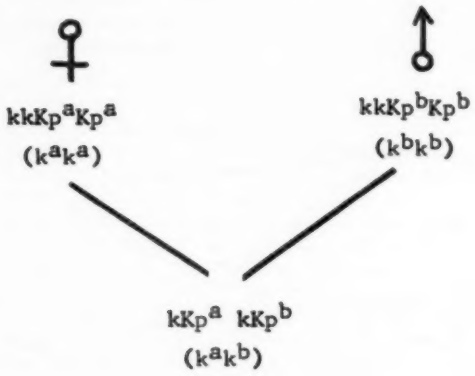

Figure 3
Case of Mrs. A. B.

The second example of Anti-Rautenberg was found at the Presbyterian Hospital, New York City in the serum of a Mrs. M. N. in 1957, by Scudder, Sargent, Race, Sanger, Cahan and Jack.[6] It is believed, as in the original Rautenberg, that Mrs. M. N. became immunized for Anti-Rautenberg, as a result of transfusion. (See Figure No. 4.)

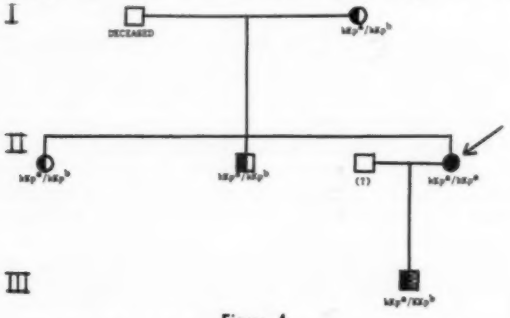

Figure 4
Case of Mrs. M. N. (Anti-Rautenberg + Anti-Kell)
(Scudder, Sargent, Race, Sanger, Cahan and Jack—1957)

In 1951, many years after the birth of her only son, Mrs. M. N. received 14 transfusions with no apparent difficulty. Upon readmission in 1956 however, she reacted with chills and temperature rise to each of four transfusions received over a two week period.

The serum of Mrs. M. N. also contains a strong Anti-Kell, as did the original Mr. Rautenberg. The presence of the Anti-Kell was established early in the investigation, when absorption with a Kell negative, Rau-

tenberg positive cell, left it as a specific antibody in the serum. Whether the Anti-Kell resulted from transfusion or from pregnancy (her son is Kell Positive) is not known. On the basis of probability however, it is assumed by the authors that the Anti-Rautenberg did result from the many transfusions in 1951, all of which, more than likely, were Rautenberg positive.

The third example of Anti-Rautenberg differs from these two, in that it is "pure" Anti-Rautenberg and is, for the first time, associated with hemolytic disease of the newborn, rather than transfusion. (See Figure No. 5).

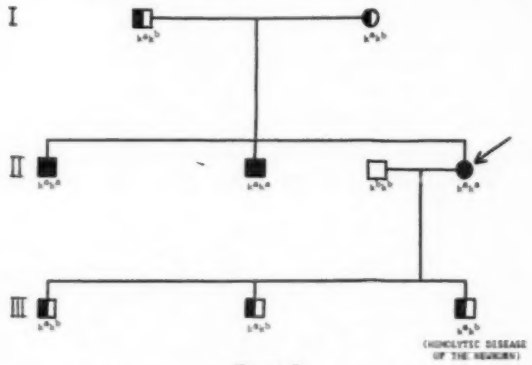

Figure 5
Case of Mrs. A. B. ("Pure" Anti-Rautenberg)

The serum of the propositus, Mrs. A. B. was sent to the Knickerbocker Foundation, New York by Mrs. Lois Anderson and Dr. J. B. White of the Terrell's Laboratories Blood Bank, All Saint's Hospital, Fort Worth, Texas. The patient had a history of two previous deliveries which were normal and uncomplicated. Further, there was no history of blood transfusion or injection of whole blood.

The patient was group A_1 Rh positive.

In July, 1958, she delivered at term, her third child which was group O, Rh positive.

The baby at birth had a strong 4+ positive direct Coombs Test and a bilirubin of 3.5 mgms per 100 cc. that rose within 8 hours to 8.9 mgms. The baby was jaundiced and by the second day the bilirubin rose to 16.6 mgms. per 100 cc. and then slowly began to decrease. He recovered spontaneously without benefit of exchange transfusion, but when last tested at 3 months of age, still gave a 1+ direct Coombs Test reaction.

Examination of the serum of Mrs. A. B. with Panocell,[R] showed strong positive reactions against all cells tested except her own. An eluate prepared from the cord blood also agglutinated all cells with the exception of the mother's, demonstrating in fact, that the same specific antibody was coated on the baby's red cells.

A clue to the identity of this antibody occurred when the cells of the propositus were tested with the two known Anti-Rautenberg sera, and gave negative results. She was in fact Rautenberg negative.

Family studies revealed the first generation parents were both of the type k^ak^b. Interestingly, their 3 children, the propositus and her two brothers, are all of the type, k^ak^a.

The propositus married a man who is of the type k^bk^b and of course, their 3 children follow expected inheritance and are all k^ak^b. The first two children born were quite normal, and only the third child presented the symptoms of mild hemolytic disease.

Titration studies of the Anti-Rautenberg in the serum of the propositus showed definite dosage effects when tested against homozygous and heterozygous Rautenberg positive cells, being 1:256 with the former and 1:64 with the latter.

Because the two previously reported Anti-Rautenberg sera both contained Anti-Kell, absorption studies were done to determine if this serum had anti-Kell or any other abnormal antibody. Absorptions with Kell negative Rautenberg positive cells removed all antibodies from the serum.

No laboratory or clinical evidence could be found that would account for the baby's hemolytic disease other than the demonstrated Anti-Rautenberg antibody.

The case of Mrs. A. B. is instructive for it shows that hemolytic disease of the newborn can result from pure Anti-Rautenberg. This antibody had been previously associated only with transfusion reactions.

In summary, two cases involving Anti-Rautenberg have been presented. One resulted from multiple transfusion and was accompanied by a strong Anti-Kell; while the other was associated, for the first time, with hemolytic disease of the newborn, and was a pure Anti-Rautenberg.

We wish to thank Dr. Fred H. Allen for gifts of rare Penney and Rautenberg serum and cells, and Drs. Robert R. Race and Ruth Sanger Race for their collaboration in confirming some of these tests.

REFERENCES

1. Allen, F. H. Jr., and Lewis, S. J. (1957). "Kp^a (Penney), a new antigen in the kell blood group system." Vox Sang. **2,** 81-87.
2. Allen, F. H. Jr., Lewis, S. J., and Fudenberg, H. (1958). "Studies of anti-Kp^b, a new antibody in the kell blood group system." Vox Sang. **3,** 1-13.
3. Coombs, R. R. A., Mourant, A. E., and Race, R. R. (1946). "In-vivo isosensitization of red cells in babies with hemolytic disease." Lancet, i, 264-266.
4. Choun, B., Lewis, M., and Kaita, K. (1957). "A 'new' kell blood-group phenotype." Nature, **180,** 711.
5. Levine, P., Backer, May, Wigod, M., and Ponder, Ruth (1949). "A new human hereditary blood property (Cellano) present in 99.8% of all bloods." Science, **109,** 464-466.
6. Scudder, J., Sargent, M., Race, R. R., Sanger, R., Cahan, A., and Jack, J. A. Unpublished observations.

THE CLINICAL SIGNIFICANCE OF SPINAL FLUID EXAMINATION*

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The cerebrospinal fluid is the equivalent of tissue fluid in other parts of the body and, therefore, because it comes in intimate contact with every part of the central nervous system, its examination provides a great deal of clinical information.

The spinal fluid is obtained in three different manners. The commonest way is to perform a lumbar puncture, usually between the third and fourth lumbar vertebrae and thus obtain spinal fluid from the lumbar subarachnoid space. Secondly, the spinal fluid may be obtained from the lateral ventricles of the brain but since this requires making an opening in the bony skull, this is usually carried out by neurosurgeons in special circumstances. A third manner of obtaining the spinal fluid is through a puncture into the cisterna magna. The spinal fluid in those three areas is essentially the same in composition, except for the total protein which is higher in the lumbar area than it is in the cistern, and which in turn is higher in the cistern than it is in the ventricles.

The normal spinal fluid is completely colorless and crystal clear in appearance. There are no red cells in it and leucocytes should not exceed five per cubic millimeter. The sugar content is best defined as being between sixty and eighty percent of a simultaneously determined blood sugar. This is particularly important since many textbooks give the sugar content as an absolute value. This is misleading since blood sugars may vary considerably depending on glucose intake and thus the definition of the normal spinal fluid sugar as a percent of the blood sugar is much more valuable. The total protein varies considerably in different laboratories due to minor differences in techniques and equipment and is usually between fifteen and sixty milligrams percent. The colloidal gold curve should be flat although a reading of 1 or 2 is still considered within normal limits. The serology, of course, should be negative.

The Normal CSF

APPEARANCE—Colorless and crystal clear
CELLS: Erythrocytes—none
Leukocytes—0—5/cu. mm.
SUGAR: 60-80% of simultaneously determined blood sugar
PROTEIN: 15-60 mg.% (varies in different laboratories)
COLLODIAL GOLD CURVE: 0000000000 (any tube may be 1 or 2)
SEROLOGY: Negative

Blood may be found in the spinal fluid as the result of two causes. One, it may have penetrated there as a result of a hemorrhage within the central nervous system which has leaked to the subarachnoid space. Secondly, the blood may have been introduced at the time of the spinal, cisternal or ventricular tap and we then speak of a traumatic spinal fluid. It is, of course, extremely important to determine the origin of this blood and the best way to do this is to centrifuge the spinal fluid im-

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Read at the Ninth Annual Postgraduate Course for Medical Technologists.

mediately and determine the color of the supernatant fluid. In hemorrhage, which is of more than two hours duration, a slight yellowish discoloration will be present and the fluid is then said to be xanthochromic. In traumatic or "bloody" tap, the supernatant will be completely clear. Other causes for xanthochromia of the spinal fluid exist and will be covered subsequently. It is frequently stated that one way of differentiating between the two is by the fact that in true hemorrhage, the red cells should be crenated, while in blood taps, the red cells will not be crenated. This is fallacious since an interval of time sufficient for crenation of cells will usually intervene between the spinal tap and CSF examination in the laboratory. Another way is to count the red cells in three different tubes obtained serially at the time of spinal tap, since in a traumatic tap it will frequently occur that the cell count will be higher in the first than in the second and than in the third tube. A useful formula for determining how much of the spinal fluid protein, and how many of the spinal fluid white cells are simply the result of blood having been brought into the subarachnoid space by the spinal needle, is given in Table 2.

Blood in the CSF

Color: From opalescent to frankly bloody.
Differentiate traumatic tap from true subarachnoid hemorrhage: Centrifugation of fluid.
Traumatic Tap—Clear, colorless supernatant
True Hemorrhage—Xanthochromic supernatant
In traumatic tap or very early hemorrhage
750 RBCs = 1 Mg.% of CSF protein
= 1 WBC in CSF

Xanthochromia of the spinal fluid may be the result of several conditions which have been listed in Table 3. The xanthochromia, seen in spinal fluids containing high levels of protein, has never been satisfactorily explained since this is far from being directly related to the total amount of protein. Thus, in some patients with a high protein, the fluid will be colorless, while in others with protein not quite as high, the fluid will be colored yellow. It should be emphasized that when examining the spinal fluid for color such as xanthochromia, it is important to hold the tube against a perfectly white background.

CSF Xanthochromia

Hemorrhage (hemoglobin to bilirubin)
Severe Jaundice
Leptospiral Meningitis (Weil's disease)
Very high CSF protein (subarachnoid block, polyneuropathies, chronic meningitis, some brain tumors
Metastatic melanoma to CNS

In almost all infections of the central nervous system (Table 4) the two most important components of the spinal fluid are the white cells and the sugar level. A table reproducing the CFS formula in infections reveals that in bacterial, (pyogenic) meningitides, the predominant leucocyte form is the polymorph, while in viral chronic meningitides the predominant form is the lymphocyte. It should be pointed out that in very early tuberculous or fungal meningitis, polymorphs may pre-

dominate so that the significance of the leucocyte form found in the spinal fluid in infections is dependent upon the time at which the spinal tap was performed in relation to the onset of the infection. In addition to the determination of cells and sugar, (and protein which is really not important) smears and cultures are, of course, of great importance. A Gram stain will promptly identify most pyogenic organisms, while if acid fast or fungal infection is suspected, special types of smears should be used. In particular, if torula (crytococcus) is suspected, an India ink smear should be done, since the organism will be readily identified by its clear halo-like capsule which will stand out against the black background. Occasionally, during the examination of the spinal fluid cells, one can find that some of the cells present are actually neoplastic cells which have become detached from a tumor or that some of them are mistaken for the organisms of torulosis. Animal inoculation should also be done and the choice of the animal depends upon the organisms suspected. Guinea pig is indicated for tuberculosis while a mouse is the animal of choice for torulosis. Repeated examination of the spinal fluid is an extremely important means of determining the progress of therapy in infections of the central nervous system.

THE CSF FORMULA IN INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

	Appearance	Cells	Sugar	Protein
Bacterial (pyogenic)	Cloudy	Polys (200-1000)	Very Low	Elevated
Viral Aseptic Abscess	Clear	Lymphocytes (100-200)	Normal	Normal or sl. elevated
Tuberculous Fungal	Varies	Lymphocytes (50-100)	Low	Elevated

Additional Tests Necessary:
Smear: Gram (India ink for Torulosis)
Culture routine, for A.F.B.
For fungi = Sabouraud's medium
Animal innoculations—guinea pig for A.F.B.
—mouse for torulosis

Chemical examination of the spinal fluid usually consists of determinations for sugar, chlorides and total protein. In recent years it has been realized that a number of conditions will lower spinal fluid sugar in addition to the acute bacterial and tuberculous meningitides which had been known for a long time. Table 5 lists these conditions which should, therefore, be considered in a differential diagnosis of low spinal fluid sugar. Again, it should be emphasized that a "low" spinal fluid sugar means that it is low when compared to a simultaneously determined blood sugar.

Hypoglycorrachia

1. In acute bacterial meningitides.
2. In hypoglycemia from any cause.

3. In tuberculous meningitis.
4. In fungal meningitis.
5. In carcinomatous meningitis.
6. In sarcomatous meningitis.
7. In gliomatous meningitis.
8. In leukemic infiltration of the meninges.
9. In Boeck's sarcoid of the CNS.

In the past, a great deal of attention has been paid to the chloride measurement in the spinal fluid especially in cases of tuberculous meningitis. Nowadays, this determination has lost a great deal of its popularity since it has been shown that the spinal fluid chloride level simply reflects the blood chloride level as modified by the Donnan equilibrium.

The determination of the total protein content is of special importance in the diagnosis of a number of conditions of the central nervous system. Table 6 lists the conditions in which the protein may be elevated. While it has been stated that the spinal fluid protein is elevated in brain tumors, it should be realized that many brain tumors occur without elevation of the spinal fluid protein.

Elevation of CSF Total Protein

1. Always in complete spinal block (tumor).
2. Almost all infections of CNS—Late in viral infections, frequently in brain abscess, frequently in **active** CNS syphilis.
3. Frequently in polyneuropathies of all types (**Very** high in Guillain-Barre syndrome).
4. Frequently in brain tumors.
5. Occasionally in subdural hematoma.
6. Occasionally in rapid, severe cerebral atrophy.
7. Occasionally in multiple myeloma.
8. Occasionally in acute "allergic" inflammatory reactions=multiple sclerosis, acute disseminated encephalomyelitis.
9. In xanthochromic fluid from hemorrhage.

There are many special tests that can be done on the spinal fluid and the choice of some of these tests depends upon individuals and laboratories. In general, a serological examination is performed and the presence of a positive serology is usually taken as an indication of involvement of the central nervous system by syphilis. It should be pointed out, however, that the presence of a positive serology is not an indication of "activity" of the disease since this has to be determined by the protein and cells that are present. In other words, a patient who has had syphilis of the central nervous system and has been adequately treated would probably continue to have a positive serology in his spinal fluid for the rest of his life. It should also be pointed out that the Treponema Immobilization Test (T. P. I.) can be performed on the spinal fluid where its accuracy is comparable to the one performed in blood. The spinal fluid serology may be negative in one form of CNS syphilis which has not been treated: in tabes dorsalis, the spinal fluid serology may be negative in cases which are "burned out." Serological tests should never be performed on spinal fluid containing blood, since such a test will

simply reflect the blood serology and have no significance in terms of syphilitic involvement of the central nervous system.

The colloidal gold curve is of value in cases of syphilis of the central nervous system, and of special value in cases in which multiple sclerosis is suspected. Thus in the absence of a positive serological test for syphilis in the spinal fluid, a positive colloidal gold curve, (with at least one tube having a 3 reading) is very strong evidence in favor of the diagnosis of multiple sclerosis. During the last few years the determination of the gamma globulin content of the spinal fluid by a number of methods has been considered even more valuable evidence in the diagnosis of multiple sclerosis. The gamma globulin will also be elevated in cases of CNS syphilis but a positive CSF serology will easily establish that diagnosis.

In summary, it should be pointed out that it is important that all tests be done on the spinal fluid with accuracy and circumspection since this material is not as easily obtained as blood, and every drop of it should be utilized to the utmost. In many diseases of the central nervous system the most valuable and, occasionally, the only information indicative of diagnosis and possible therapy is determined by a careful examination of the cerebrospinal fluid.

Wanted at once: Medical Technologist (ASCP certified or eligible) for general hospital, 100-beds. Moving to new 150-bed hospital 1960. 5 Technologist Laboratory. Attractive salary and fringe benefits. Near beaches. Contact Miss Helen Chappell, MT (ASCP), Arbemarle Hospital, Elizabeth City, North Carolina.

Medical Technologist, ASCP or eligible, for a 300 bed general hospital, rotation on every ninth night with liberal compensation time off, one month vacation, holidays and sick leave. Salary $325-$400 with annual increases. Approved school, New Jersey seashore resort area. Contact: Pathologist, Fitkin Memorial Hospital, Neptune, N. J.

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ASCP Registered Medical Technologists—$420 per month to start. Need four well qualified Medical Technologists by September 1, 1959. Write: Daniel F. Glaser, M.D., Pathologist, Bronson Methodist Hospital, Kalamazoo, Michigan.

If you are a registered medical technologist (ASCP) and enjoy employment in a general hospital actively engaged in teaching and research programs, good personnel policies with attractive salaries, 40 hour week—Contact:

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THE METHODIST HOSPITAL

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6516 Bertner Ave.
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(Continued on Page XXXII)

THE THEORY OF THE PAS METHOD OF STAINING*

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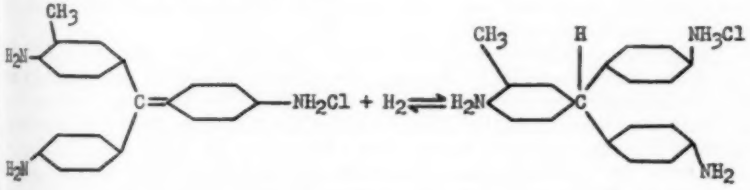
With the increasing frequency of fungus infections, the Periodic Acid-Schiff (PAS) method of staining fungi in tissues is of tremendous value in the Histological Laboratory. It has been stated that this procedure may become a diagnostic method in the routine laboratory.

The whole of modern histochemistry of polysaccharides, muco-polysaccharides, and mucoproteins is bound up with the Periodic Acid-Schiff Reaction. This reaction involves the oxidation of adjacent hydroxyl groups to aldehydes.

The process of oxidation-reduction involves a change in the arrangement of the electrons of an atom. The reaction in which an atom gives electrons is termed oxidation; while the reaction in which an atom gains electrons is termed reduction. Oxidation and reduction are mutually dependent processes—it is obvious that if electrons are taken up by one substance, they must be given up by another. There can be no oxidation without reduction, and no reduction without oxidation taking place simultaneously.

Certain definite atomic groupings, known as chromophores, are responsible for the colored compounds in which it occurs. Some of the chromophores have a basic character, others acidic. These chromophores differ considerable, but they have one property in common—they all have unsatisfied affinities for hydrogen; or, in other words, they are all easily reducible. This reduction destroys the chromophore groups, and as a result the compound loses its color. These colorless compounds are known as leuco compounds. Thus, fuchsin yields leuco-fuchsin on reduction. Fuchsin, being reduced through the action of sulphite, has the following general type of formula:



This formula is not exactly that of Schiff's Reagent, as it is now believed that the sulphite radical in some way enters into its composition.

This compound of basic fuchsin and sulfurious acid is regarded as a micro-chemical reagent for detecting the presence of aldehyde-like substances with the resulting formation of a purple colored compound.

The periodic acid, as an oxidant, breaks the C—C bonds in the various structures in which they are present as 1 : 2-glycol groups, converting them to dialdehydes. The equivalent amino or alkylamino derivatives of 1:2-glycol or its oxidation product are also attacked and converted to

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dialdehydes. The oxidation takes place according to the equation:

$$C\,HOH - C\,HOH + H_5IO_6 \rightarrow C\,HO - C\,HO + HIO_3 + H_2O$$

The particular property of periodic acid, which renders it superior to other reagents commonly used in histochemistry for oxidation of C C bonds, ($KMnO_4$, H_2CrO_4, H_2O_2), is that it does not further oxidize the resulting aldehydes. These can, therefore, be localized by combination with Schiff's Reagent to give a substituted dye. The dye is somewhat violet in color, rather than red. After contact of the leuco-fuchsin with the aldehyde-like constituents of the cells, some chemical reaction takes place which is not wholly understood. This chemical reaction evidently restores the quinonoid structure of the molecule, and, accordingly, the color of the compound. The violet color, rather than red, seems to indicate that some other chemical change in the dye takes place in addition to reduction. As previously mentioned, it is now believed that the sulphite radical combines with the reduced compound in some way. The resulting compound, Schiff's Reagent, is now generally called "fuchsin-sulfurous acid" rather than leuco-fuchsin. It must be understood that this dyestuff is a new compound and not re-oxidized fuchsin. Many basic fuchsins contain an impurity which does not completely decolorize under the action of sulphite; therefore, a decolorizing carbon is used to eliminate this impurity from a decolorized fuchsin.

The amount of color developed by the Schiff's PAS reaction is dependent upon the amount of reactive glycol structure present in the tissue concerned. According to Hotchkiss (1948), positive results are given by any substance which fulfills the following four criteria:

1. Contains the 1:2-glycol grouping or equivalent amino or alkylamino derivative, or oxidation product C—HOH—CO;
2. Does not diffuse away in the course of tissue fixation;
3. Gives an oxidation product which is diffusible; and
4. Is present in sufficient concentration to give a detectable final color.

It appears that only the high molecular substances are likely to be present in sufficient quantity to give a positive result.

The chief substances in animal tissue which exhibit the stain following the reaction of the PAS stain are glycogen, mucin, mucoproteins, and presumably hyaluronic acid and chitin. The pentoses of nucleic acid are so substituted that they will not give the reaction and cerebrosides, if present, would be expected to react.

It must be realized that differences in the PAS reaction of given structures are as much due to variation in technique as to variation in the structures themselves. The results may vary, depending upon the species from which they are derived and on the type of fixative employed. The varying of the solvent used for the periodic acid and the time of oxidation are also to be considered.

The specificity of the Feulgen reaction for aldehydes has been brought up repeatedly. Oster and Oster (1946) have examined the question of specificity and have found that the "true" reaction is indeed specific for aldehydes; whereas other carbonyl compounds giving a "pseudo" reaction may be detected on the basis that the purple color developed in the "true" reaction can be decolorized with dilute sodium hydroxide and restored to its original intensity with hydrochloric acid, while the

reddish color of the "pseudo" reaction cannot be restored by acid after decolorization.

Summary

Periodic acid is an oxidant potent enough to break the C-C bond and oxidize aldehyde groups.

The Periodic Acid-Schiff Reaction involves the oxidation of adjacent hydroxyl groups to aldehydes and depends on the formation of a purple-colored compound when these aldehydes react with the fuchsin-sulfurous acid. The chemical reactions leading to the staining are rather well known, and the outcome, in general, predictable, if there is knowledge of the chemical structure.

REFERENCES

1. Glick, D.: *Techniques of Histo and Cytochemistry;* Interscience Publishers, New York; 1949.
2. Pearse, A. G. E.: *Histochemistry, Theoretical and Applied;* Little Brown and Company, Boston; 1953.
3. Conn, H. J.: *Biological Stains;* Biotech Publications, N. Y.; 1954.
4. Bangle, R., Jr.: The Peracetic Acid-Schiff Stain; Amer. Jour. Cl. Path., 24: 178-185; 1954.
5. Hotchkiss, R. D.: A Microchemical Reaction Resulting in the Staining of Polysaccharide Structures in Fixed Tissue Preparations; Arch. of Biochem.; 16: 131-141; 1948.

Acknowledgment

The author wishes to acknowledge with sincere gratitude the interest, encouragement, and suggestions of Louise Stinson, M.S., MT (ASCP).

CHECK LIST OF SCIENTIFIC JOURNALS AND ALLIED PERIODICALS FOR THE CLINICAL LABORATORY*

JOHN S. HANNAN, B.S., MT (ASCP)
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The following list is presented for the convenience of those who may wish to expand their supply of current literature. It is based on first-hand knowledge of virtually all the publications included.

Six main groups are designated:

Journals of Definite Interest
Journals of Probable Interest
Journals of Possible Interest
Abstracts
Indices
Popular Periodicals

JOURNALS OF DEFINITE INTEREST

The Journal of Medical Laboratory Technology in addition to original articles, contains a large number of abstracts under the headings of Bacteriology, Mycology and Virology; Chemical Pathology (Clinical Chemistry); Hematology and Blood Transfusion Technic; Histopathology and is Great Britain's equivalent of *The American Journal of Medical Technology*. The Institute of Medical Laboratory Technology, 74, New Cavendish Street, Harley Street, London, W. 1, England. Quarterly.

Abstracts of Bioanalytic Technology presents selected articles in the biological sciences more or less directly bearing on the laboratory study of human health and disease. The aim is to offer abstracts in a form which will permit individual readers to judge the importance to be attached to the original article, includes material from 1200 English and foreign language journals. Whenever possible, sufficient detail is included to allow preliminary trial of new procedures without requiring reference to original publications. Sections: Bacteriology, Chemistry, Endocrinology, Hematology, Mycology, Parasitology, Serology, Virology. Published quarterly by the American Association of Bioanalysts, Rm. 1202, 7 West Madison Street, Chicago 2, Illinois. (Although in abstract form, this periodical is included in this group because of its particular nature).

The above two publications cannot be praised too highly, and should be in every laboratory. *The Journal of Medical Laboratory Technology* contains a number of concise, pertinent articles. The abstracts, under References to Publications, afford an excellent view of current work in all phases of medical technology, as well as serving to point out articles which one may choose to read in entirety.

The technics provided by *Abstracts of Bioanalytic Technology* are well-chosen and will save considerable searching through the literature. One important advantage of both periodicals is that they include information gleaned from journals and other sources not ordinarily associated with the clinical laboratory.

The Canadian Journal of Medical Technology official publication of the Canadian Society of Laboratory Technologists, 61 Victoria Ave.,

* Received for publication December 1958.

North, Hamilton, Ontario, Canada; the counterpart of *The American Journal of Medical Technology.*

Medical Technicians Bulletin Supplement to *U. S. Armed Forces Medical Journal.* Supt. of Documents, U. S. Govt. Printing Office, Washington 25, D. C.

Journal of Clinical Pathology includes a number of articles of primary interest to the technologist, as well as a section of Technical Methods. British Medical Association, Tavistock Square, London, W. C. 1, England.

The Journal of Laboratory and Clinical Medicine contains a section of Laboratory Methods. C. V. Mosby Co., 3207 Washington Blvd., St. Louis 3, Missouri.

Clinical Chemistry Journal of the American Association of Clinical Chemists. 3110 Elm Ave., Baltimore 11, Maryland.

The Scandinavian Journal of Clinical & Laboratory Investigation All articles are in English. E. Munksgaard, Norregade 6, Copenhagen, Denmark.

Technical Bulletin of the Registry of Medical Technologists a publication is listed for the benefit of those who are not members of The American Society of Medical Technologists. Williams & Wilkins Co., Mt. Royal and Guilford Aves., Baltimore 2, Maryland.

The Laboratory Digest magazine-type, reports the topics of Antibiotics, Atomic Medicine, Bacteriology, Biochemistry, Biophysics, Entomology, Hematology, Immunology & Serology, Mycology, Parasitology, Pathology, Forensic Pathology, Surgical Pathology, Public Health, Sanitation and Food Microbiology, Toxicology, Tropical Medicine, Virology. Charles Wedemeier, Business Manager, 3514 Lucas Avenue, St. Louis, Missouri.

JOURNALS OF PROBABLE INTEREST

Blood The Journal of Hematology. May be listed as **Blood,** N. Y. Grune & Stratton Inc., 381 Fourth Ave., New York 16, New York.

British Journal of Haematology Charles C. Thomas, Publisher, 301-327 East Lawrence Ave., Springfield, Illinois.

Acta Haematologica International Journal of Hematology. Papers in English, German or French with summaries of about 100 words in each of these languages. S. Karger, Basel, Switzerland. American agent: Mr. Albert J. Phiebig, P. O. B. 352, White Plains, New York.

Vox Sanguinis* Journal of Blood Transfusion and Immunohematology. All articles in English. S. Karger, Basel, Switzerland. American agent: Mr. Albert J. Phiebig, P. O. B. 352, White Plains, New York.

Problems of Hematology and Blood Transfusion Pergamon Press, 122 E. 55th St., New York 22, New York.

Stain Technology Williams & Wilkins Co., Mt. Royal & Guilford Aves., Baltimore 2, Maryland.

Tubercle (Journal of the British T. B. Association). Staples Press Limited, 3 Mandeville Place, London, W. 1, England.

Annals of Tropical Medicine and Parasitology Liverpool University Press, 123 Grove Street, Liverpool 7, England.

*Perusal of additional issues of Vox Sanguinis reveals that most, but not all articles are in English.

JOURNALS OF POSSIBLE INTEREST

Journal of the Royal Microscopical Society Royal Microscopical Society, Tavistock House South, Tavistock Square, London, W. C. 1, England.

Archives of Disease in Childhood A certain proportion of articles pertain to laboratory procedures. British Medical Association, Tavistock Square, London, W. C. 1, England.

Therapeutic Notes Has a short section under the heading of Laboratory Procedures in Office Practice. Distributed to physicians by Parke, Davis & Co., Detroit 32, Michigan.

Bulletin of the World Health Organization While only an occasional article is of direct interest to the laboratory, these are very comprehensive. A series of studies on the laboratory diagnosis of various diseases is being prepared which, it is hoped, eventually will be revised and published in monograph form. An effort is being made to ensure that the diagnostic methods recommended are as internationally representative and acceptable as possible by securing the co-operation of a number of experts from different countries. This promises to be a most valuable reference work. Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York.

Postgraduate Medicine, Minneapolis Not primarily a laboratory journal, but contains Laboratory Notes by Dr. A. H. Sanford. Postgraduate Medicine, Essex Building, Minneapolis 3, Minnesota.

Proceedings of the Royal Society of Medicine The Royal Society of Medicine, 1 Wimpole Street, London, W. 1, England. U. S. agent: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, New York.

Journal of Forensic Sciences Callaghan and Co., 6141 North Cicero Ave., Chicago 30, Illinois.

South African Journal of Laboratory and Clinical Medicine Not many articles are directly applicable to the laboratory as known in the United States. Medical Association of South Africa, P. O. Box 643, Capetown, South Africa.

Laboratorio Entirely in Spanish, includes Bacteriology, Immunology, (Parasitology, Hematology, Pathological Anatomy, and Clinical Chemistry. Gran Via, 19 Moderno, Granada, España (Spain).

Revista Cubana de Laboratorio Clinico* Practically all articles are in Spanish; during 1957, there were two in English. Sociedad Cubana de Médicos Laboratoristas Clínicos, San Lázaro No. 60, altos, La Habana, Cuba.

ABSTRACTS

Although not intended to replace original articles, abstracts offer a means of surveying a very large field of publications. Since these abstracts translate articles from foreign languages, much otherwise unavailable information may be obtained.

Analytical Abstracts Sections of Blood, Bile, Urine, etc., General Technic and Laboratory Apparatus. Secretary, The Society for Analytical Chemistry, 14, Belgrave Square, London, S. W. 1, England.

Excerpta Medica (International Abstracting Service)
Section VI INTERNAL MEDICINE Includes Apparatus, Biochemical and Physico-chemical Tests, Micro-

* The address of the Revista Cubana de Laboratorio Clinico has recently been changed to Edificio Medico, 23 yN (40 piso-) Vedado, Habana, Cuba.

scopical Diagnostics, Parasitology, Blood-Hemopoietic System.

Section IV MEDICAL MICROBIOLOGY, IMMUNOLOGY & SEROLOGY

Section II PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY Includes Apparatus & Technics, Clinical Analysis, Body Fluids.

Sections are obtainable separately. Excerpta Medica Foundation, The N. Y. Academy of Medicine Bldg., 2 E. 103rd St., New York 29, New York. (Also local distributors and in many other countries.)

Abstracts of World Medicine A monthly critical survey of periodicals in medicine and its allied sciences. Portions of interest might include Chemical Pathology, Hematology, Microbiology and Parasitology, Tuberculosis, Venereal Diseases, Tropical Medicine, Clinical Hematology. More than 1600 periodicals are surveyed, from which are selected for abstracting those papers which appear to make some useful contribution to the sum of medical knowledge or or experience. British Medical Association, Tavistock Square, London, W. C. 1, England.

Tuberculosis Index Includes chest diseases. National Association for the Prevention of Tuberculosis, Tavistock House North, London, W. C. 1, England.

Tropical Diseases Bulletin Contains abstracts of papers (from all parts of the world) dealing with:

Protozoal infections (malaria, trypanosomiasis, leishmaniasis, and others)
Rickettsial diseases
Tropical virus diseases (yellow fever and others)
Tropical bacterial disease (plague, cholera, dysentery, relapsing fever, yaws, leprosy and others)
Helminthic diseases
Other conditions related to warm climates
Bacteriological, entomological, protozoological papers related to these diseases

Bureau of Hygiene and Tropical Diseases, Keppel Street, London, W. C. 1, England.

INDICES

Used much in the manner of the index to a book, the indices provide a means of obtaining comprehensive information.

Current List of Medical Literature Soon after articles are published in the various journals their titles may appear in this list. Some articles in foreign languages are included. The titles are listed according to subject matter; each title being numbered to direct the reader to the particular journal in which the article may be found. Also, each issue of each journal included is listed with the titles contained therein. Supt. of Documents, U. S. Govt. Printing Office, Washington 25, D. C.

Quarterly Cumulative Index Medicus This publication is somewhat similar to the above, with the exception that it appears several years later. It has two sections, one concerned with books and the other with periodical literature. The book section includes publications alphabetized as to authors, followed by a subject classification of the same material. In the periodical section, articles are listed by subject, with numerous

cross references to assist in locating tests, etc. There is also a list of publishers and a list of journals indexed. American Medical Association, 535 N. Dearborn St., Chicago 10, Illinois.

POPULAR PERIODICALS

The following two publications, apart from their intrinsic interest, are well-suited for the waiting room or similar situations.

Scientific American Scientific American, Inc., 415 Madison Ave., New York 17, New York.

Today's Health Today's Health, American Medical Association, 535 N. Dearborn St., Chicago 10, Illinois.

In addition to subscribing, publications in any of the above categories may be obtained in a number of ways. Certain libraries, of course, will contain many. The library of the New York Academy of Medicine in New York City, for example, is open to the public. In Chicago, The John Crerar Library offers a Medical Department and a Technology Department.

All items listed in the *Current List of Medical Literature* are available at the National Library of Medicine in Washington, D. C., and these should include practically every journal required. Readers who cannot obtain the journals at local libraries and who cannot come to the National Library of Medicine in person may use the inter-library loan services of the National Library of Medicine, which are designed to extend the services of the library at a distance. Details may be found in the inside cover of the *Current List of Medical Literature*. Individuals must route their requests through a library.

Approximately 200 journals—some in foreign languages—are available on loan from the library of the American Medical Association (535 N. Dearborn St., Chicago 10, Illinois). This service is available to individuals of the United States and Canada who subscribe to the scientific periodicals of the American Medical Association and to members of the American Medical Association and its student organizations. There is no charge to members, but there is a nominal fee for other borrowers. Three journals may be borrowed at one time for a period not exceeding five days. Further information, including a list of journals available, may be found in the front of Vol. 162[1] of *The Journal of the American Medical Association*, Sept.-Dec., 1956. The *American Medical Association Archives* cannot be lent. The journals in the present check list which may be borrowed are:

Archives of Disease in Childhood
Blood, N. Y.
Journal of Clinical Pathology
The Journal of Laboratory and Clinical Medicine
Postgraduate Medicine, Minneapolis
Tubercle

To the newcomer to scientific literature, it should be pointed out that for a given individual the articles will probably be of various degrees of interest. As a result, in the case of some journals, it may be that only a few articles each year are given full consideration. Back issues are well worth study; scientific information does not ordinarily become outdated quickly. In some instances a certain amount of historical development is necessary to completely understand current work.

Several types of binders and cases are obtainable* for assembling back issues into a neat and orderly reference file.

* Demco Library Supplies (catalog available), Box 1070, Madison, Wisconsin, or Box 1772, New Haven, Connecticut.